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(54) Title: POLYNUCLEOTIDES AND POLYPEPTIDES ASSOCIATED WITH ANTIBIOTIC BIOSYNTHESIS AND USES THEREFOR

(57) Abstract: The present invention discloses polyketides and the polyketide synthases and ancillary enzymes that are capable of producing such compounds. More particularly, the present invention discloses polynucleotides and polypeptides associated with (i) a novel polyketide synthase linked to a non-ribosomal peptide synthetase involved in the biosynthesis of albicidins, (ii) a novel phosphopantetheinyl transferase for activating enzymes, particularly polyketide synthases and/or non-ribosomal peptide synthetases, associated with the biosynthesis of albicidins, and (iii) a novel methyltransferase for methylating precursors of albicidins and/or intermediates related to albicidin biosynthesis. The present invention also discloses methods of using the aforementioned polynucleotides and polypeptides for activating polyketide synthases and/or non-ribosomal peptide synthetases, for methylating precursors of albicidins or their analogues and/or intermediates involved in the biosynthesis of albicidins or analogues thereof and for enhancing the level and/or functional activity of albicidins or their analogues. Also disclosed are methods of using the polynucleotides and polypeptides of the invention for the biosynthesis of albicidins or their analogues.

POLYNUCLEOTIDES AND POLYPEPTIDES ASSOCIATED WITH ANTIBIOTIC BIOSYNTHESIS AND USES THEREFOR

FIELD OF THE INVENTION

THIS INVENTION relates generally to antibiotic biosynthesis. More particularly, the present invention relates to polyketides and the polyketide synthases and ancillary enzymes that are capable of producing such compounds. Even more particularly, the present invention relates to a polyketide synthase linked to a non-ribosomal peptide synthetase involved in the biosynthesis of albicidins, to a phosphopantetheinyl transferase for activating enzymes, particularly polyketide synthases and/or non-ribosomal peptide synthetases, associated with the biosynthesis of albicidins, and to a methyltransferase for methylating precursors of albicidins and/or intermediates related to albicidin biosynthesis. The present invention also relates to biologically active fragments of the aforementioned polypeptides and to variants and derivatives of these molecules. Further, the invention relates to polynucleotides encoding the said polypeptides, including the *xabA*, *xabB* and *xabC* genes of *Xanthomonas albilineans*, to polynucleotides encoding the said fragments, variants or derivatives, to vectors comprising the said polynucleotides and to host cells containing such vectors. The invention also relates to a transcriptional control element for modulating the expression of polynucleotides including, for example, the *xabB* gene and/or the *xabC* gene of *Xanthomonas albilineans*, or variants thereof. The invention also features methods of using the polynucleotides, polypeptides, fragments, variants, derivatives and vectors for activating polyketide synthases and/or non-ribosomal peptide synthetases, for methylating precursors of albicidins or their analogues and/or intermediates involved in the biosynthesis of albicidins or their analogues and for enhancing the level and/or functional activity of albicidins or their analogues. The invention also encompasses methods of using the aforesaid polynucleotides, polypeptides, fragments, variants and derivatives for the biosynthesis of albicidins or analogues thereof.

Bibliographic details of various publications referred to by author in this specification are collected at the end of the description.

BACKGROUND OF THE INVENTION

Polyketides represent a large structurally diverse group of compounds synthesised from 2-carbon units through a series of condensations and subsequent modifications. They possess a broad range of biological activities including antibiotic and pharmacological properties. For example, polyketides are represented by antibiotics such as tetracyclines, erythromycins, immunosuppressants such as FK506, FK520 and rapamycin, anticancer agents such as daunomycin and veterinary products such as monensin and avermectin.

Considering the difficulty in producing polyketide compounds by conventional chemical methodologies, and the typically low production of polyketides in wild-type cells, there has been considerable interest in finding improved or alternate means to produce polyketide compounds. In this regard, reference may be made to PCT publication Nos. WO 93/13663; WO 95/08548; WO 96/40968; WO 97/02358; and WO 98/27203; U.S. Pat. Nos. 4,874,748; 5,063,155; 5,098,837; 5,149,639; 5,672,491; and 5,712,146; Fu *et al.* (1994, *Biochemistry* **33**: 9321-9326); McDaniel *et al.* (1993, *Science* **262**: 1546-1550); and Rohr (1995, *Angew. Chem. Int. Ed. Engl.* **34**(8): 881-888).

Polyketides are synthesised in nature by polyketide synthases (PKS). These enzymes, which are actually complexes of multiple enzyme activities, are in some ways similar to, but in other ways different from, the synthases that catalyse condensation of 2-carbon units in the biosynthesis of fatty acids. Specifically, PKS enzymes catalyse the biosynthesis of polyketides through repeated (decarboxylative) Claisen condensations between acylthioesters (*e.g.*, acetyl, propionyl, malonyl or methylmalonyl). Following each condensation, they introduce structural variability into the product by catalysing all, part, or none of a reductive cycle comprising a ketoreduction, dehydration, and enoylreduction on the β-keto group of the growing polyketide chain. PKS enzymes incorporate enormous structural diversity into their products, in addition to varying the condensation cycle, by controlling choice of primer, extender units, and the overall chain length and, particularly in the case of aromatic polyketides, regiospecific cyclisation of the nascent polyketide chain. After the carbon chain has grown to a length characteristic of each specific product, it is released from the synthase by thiolysis or acyltransfer. Thus, the PKS complexes consist of families of enzymes which work together to produce a given polyketide. It is the choice of chain-building units, controlled variation in chain length, and the reductive cycle,

genetically programmed into each PKS, that contributes to the variation seen among naturally occurring polyketides.

Two major types of PKS enzymes are known; these differ in their composition and mode of synthesis of the polyketide synthesised. These two major types of PKS enzymes are commonly referred to as Type I or "modular" and Type II "iterative" PKS enzymes. These classifications are well known and reference may be made, for example, to Hopwood and Khosla (1992).

The Type I or modular PKS enzymes typically catalyse the biosynthesis of complex polyketides such as erythromycin and avermectin. These modular enzymes include assemblies of several large multifunctional proteins carrying, between them, a set of separate active sites for each step of carbon chain assembly and modification (Cortes *et al.*, 1990; Donadio *et al.*, 1991; MacNeil *et al.*, 1992). Accordingly, modular PKS complexes can be viewed as biochemical assembly lines, composed of a series of catalytic domains involved in sequential assembly and modification of acyl groups on the growing polyketide chain (Cane *et al.*, 1998; Keating and Walsh, 1999). The catalytic domains are arranged in "modules", punctuated by acyl carrier protein (ACP) domains that tether the nascent polyketide while it undergoes the catalytic modifications programmed in the associated module. For each polyketide there is an initiation module, a series of elongation modules that define the length and structure of the polyketide chain, and a termination module to release the product from the final tether. The initiation module typically comprises an acyl transferase (AT) domain that couples the initial acyl group from an acyl-CoA substrate to the phosphopantetheinyl tether of the first ACP domain. Each elongation module typically comprises a ketosynthase (KS), an AT and an ACP. The KS removes the growing polyketide unit from the upstream ACP and couples it to the next acyl group in the chain, which has already been selected and loaded by the AT onto the ACP in the same module. Other catalytic domains (*eg.* a ketoacyl reductase (KR), and dehydratase (DH)) within an elongation module can modify the newly elongated polyketide before it is transferred to the next module in the biochemical assembly line. A thioesterase (TE) domain in the termination module accomplishes release of the assembled polyketide from the last ACP in the series (Cane *et al.*, 1998; Keating and Walsh, 1999).

Biosynthesis of a polyketide can involve the sequential action of several PKS proteins, each with one to six elongation modules (MacNeil *et al.*, 1992; Apricio *et al.*, 1996). There are variations on the modular PKS design, including participation by some loading domains across modules or in *trans* from separate proteins (Keating and Walsh, 1999), and several examples of hybrid PKS/NRPS proteins (Albertini *et al.*, 1995; Gehring *et al.*, 1998; Duitman *et al.*, 1999; Paitan *et al.*, 1999). Subsequent modification of the polyketide by dedicated tailoring enzymes is generally required to complete the biologically active product (Hopwood, 1997). Other biologically active compounds including antibiotics comprise polypeptides assembled by non-ribosomal peptide synthetases (NRPSs). NRPSs typically show a modular architecture and tethered biosynthetic strategy analogous to PKSs (Cane *et al.*, 1998; Keating and Walsh, 1999). In NRPSs a condensation (C) domain removes the growing peptide unit from the upstream PCP domain and couples it to the next amino acid group in the chain, which has already been selected and loaded by an adenylation (A) domain onto the PCP in the same module (Marahiel *et al.*, 1997; Stachelhaus *et al.*, 1998). Other catalytic domains (*e.g.*, epimerase or N-methytransferase) within an elongation module can modify the newly elongated polypeptide before it is transferred to the next module in the biochemical assembly line (Marahiel *et al.*, 1997).

Many phytopathogenic bacteria and fungi secrete toxins with phytotoxic activity and a broad spectrum of antimicrobial properties (Guenzi *et al.*, 1998). Albicidin phytotoxins are polyketides produced by *Xanthomonas albilineans*, which are key pathogenicity factors in the development of leaf scald, one of the most devastating diseases of sugarcane (*Saccharum*, interspecific hybrids) (Ricaud and Ryan, 1989; Zhang and Birch, 1997; Zhang *et al.*, 1999). Albicidins selectively block prokaryote DNA replication and cause the characteristic chlorotic symptoms of leaf scald disease by blocking chloroplast development (Birch and Patil, 1983; 1985b; 1987a; 1987b). Because albicidins are rapidly bactericidal at nanomolar concentrations against a broad range of Gram-positive and Gram-negative bacteria, they are also of interest as potential clinical antibiotics (Birch and Patil, 1985a).

The major antimicrobial component of the family of albicidins produced in culture by *X. albilineans* has been partially characterised as a low M_r compound with several aromatic rings (Birch and Patil, 1985a). Low yields have slowed studies into the

chemical structure of albicidin, its application as a tool to study prokaryote DNA replication, and its development as a clinical antibiotic (Zhang *et al.*, 1998). Genetic analysis of albicidin biosynthesis is likely to indicate approaches to increase yields, probable structural features, and opportunities for engineering novel antibiotics in this family.

SUMMARY OF THE INVENTION

The present invention arises in part from the identification and characterisation of several *X. albilineans* genes associated with albicidin biosynthesis. In particular, the present inventor has isolated a novel *X. albilineans* gene (*xabB*), which encodes a large protein (predicted *Mr* 525,695), with a modular architecture indicative of a multifunctional PKS linked to a non-ribosomal peptide synthetase (NRPS). At 4801 amino acids in length, the product of *xabB* (XabB) is the largest reported PKS-NRPS. Twelve catalytic domains in this multifunctional enzyme are arranged in the order N-terminus-acyl-CoA ligase (AL)-acyl carrier protein (ACP)-β-ketoacyl synthase (KS)-β-ketoacyl reductase (KR)-ACP-ACP-KS-peptidyl carrier protein (PCP)-condensation domain (C)-adenylation domain (A)-PCP-C. The modular architecture of XabB indicates likely steps in albicidin biosynthesis, and approaches to enhance antibiotic yield. The novel pattern of domains, in comparison with known PKS-NRPS enzymes for antibiotic production, also contributes to the knowledge base for rational design of enzymes producing novel antibiotics. The present inventor has found that XabB is required for the production of albicidins and that enhanced expression of *xabB* leads to increased levels and/or functional activities of albicidin antibiotics.

A gene (*xabC*) encoding a novel *O*-methyltransferase has also been isolated, which methylates albicidin precursors and/or intermediates involved in albicidin biosynthesis. Surprisingly, enhanced expression of *xabC* has been found to increase the levels and/or functional activities of albicidin antibiotics.

The present inventor has also isolated a gene (*xabA*) encoding a phosphopantetheinyl transferase (PPTase), which is required for post-translational activation of synthetases in the albicidin biosynthetic pathway. In this regard, it is known that inefficient phosphopantetheinylation has limited the activity of other antibiotic synthetases overexpressed in heterologous species (Walsh *et al.*, 1997). Accordingly, the isolated *xabA* gene, together with its target in the albicidin biosynthetic pathway (*e.g.*, *xabB*), provide the means to engineer high level co-expression of the albicidin synthetase and its activating PPTase to obtain albicidins in higher yields, and ultimately to manipulate the elements of the albicidin biosynthetic machinery, by mutagenesis or by other means, to produce desired structural variants of this novel antibiotic class.

The above genes, in whole or in part, together with their variants and derivatives, are useful *inter alia* for modulating the level and/or functional activity of albicidins, for expressing PKS enzymes in recombinant host cells, for producing polyketides including albicidins and their analogues and for combinatorial biosynthesis, as described hereinafter.

Accordingly, one aspect of the present invention contemplates an isolated polypeptide encoding at least a portion of an albicidin PKS-NRPS (XabB) or its variants or derivatives. In one embodiment of this type, the invention provides an isolated polypeptide comprising at least one domain selected from the group consisting of:

(a) an acyl-CoA ligase (AL) domain comprising a sequence set forth in any one or more of SEQ ID NO: 6 and 8, or variants thereof.

(b) a β-ketoacyl synthase (KS) domain comprising a sequence set forth in any one or more of SEQ ID NO: 10, 12, 14, 16, 18 and 20, or variants thereof;

(c) a β-ketoacyl reductase (KR) domain comprising the sequence set forth SEQ ID NO: 22, or variants thereof;

(d) an acyl carrier protein (ACP) domain comprising a sequence set forth in any one or more of SEQ ID NO: 24, 26 and 28, or variants thereof;

(e) an adenylation (A) domain comprising a sequence set forth in any one or more of SEQ ID NO: 30, 32, 34, 36, 38, 40, 42, 44, 46 and 48, or variants thereof;

(f) a peptidyl carrier protein (PCP) domain comprising a sequence set forth in any one or more of SEQ ID NO: 50 and 52, or variants thereof; and

(g) a condensation (C) domain comprising a sequence set forth in any one or more of SEQ ID NO: 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78 and 80, or variants thereof.

Preferably, the AL domain comprises each of the sequences set forth in SEQ ID NO: 6 and 8, or variants thereof.

In one embodiment, the KS domain preferably comprises each of the sequences set forth in SEQ ID NO: 10, 12 and 14, or variants thereof. In an alternate embodiment, the KS domain preferably comprises each of the sequences set forth in SEQ ID NO: 16, 18 and 20, or variants thereof.

Preferably, the A domain comprises each of the sequences set forth in SEQ ID NO: 30, 32, 34, 36, 38, 40, 42, 44, 46 and 48, or variants thereof.

In one embodiment, the C domain preferably comprises each of the sequences set forth in SEQ ID NO: 54, 56, 58, 60, 62, 64 and 66, or variants thereof. In an alternate embodiment, the C domain preferably comprises each of the sequences set forth in SEQ ID NO: 68, 70, 72, 74, 76, 78 and 80, or variants thereof.

In another embodiment, the invention provides an isolated polypeptide comprising at least a biologically active fragment or portion of the sequence set forth in SEQ ID NO: 2, or a variant or derivative thereof.

Suitably, the biologically active fragment is at least 6 amino acids in length.

In a preferred embodiment, the domains broadly described above are arranged in an N- to C-terminal direction as follows: AL-ACP-KS-KR-ACP-ACP-KS-PCP-C-A-PCP-C.

Suitably, the biologically active fragment comprises at least one domain selected from the group consisting of the AL domain, the KS domain, the KR domain, the ACP domain, the A domain, the PCP domain and the C domain as broadly described above.

Suitably, the variant has at least 60%, preferably at least 70%, more preferably at least 80%, more preferably at least 90% and still more preferably at least 95% sequence identity to the sequence set forth in SEQ ID NO: 2.

Preferably, the variant comprises at least one sequence selected from the group consisting of SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78 and 80, or variant thereof. In this regard, the variant preferably has at least 70%, preferably at least 80%, more preferably at least 90%, and still more preferably at least 95% sequence identity to any one of the amino acid sequences set forth in SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78 and 80.

In another aspect, the present invention contemplates an isolated polypeptide encoding at least a portion of a PPTase (XabA) associated with albicidin biosynthesis or its variants or derivatives. In one embodiment of this type, the invention provides an isolated

polypeptide comprising at least biologically active fragment or portion of the sequence set forth in SEQ ID NO: 83, or a variant or derivative thereof.

Suitably, the biologically active fragment comprises at least one, and preferably both, of the consensus PPTase sequence motifs set forth in SEQ ID NO: 89 and 93, or variant thereof. Preferably, the biologically active fragment comprises the intervening sequence between the said consensus PPTase sequence motifs, which intervening sequence comprises the sequence set forth in SEQ ID NO: 91, or variant thereof.

Preferably, the biologically active fragment comprises a contiguous sequence of amino acids contained within the sequence set forth in SEQ ID NO: 87, or variant thereof.

Suitably, the variant has at least 60%, preferably at least 70%, more preferably at least 80%, more preferably at least 90% and still more preferably at least 95% sequence identity to the sequence set forth in SEQ ID NO: 83.

Preferably, the variant comprises at least one sequence selected from the group consisting of SEQ ID NO: 87, 89, 91 and 93, or variant thereof. In this regard, the variant preferably has at least 70%, preferably at least 80%, more preferably at least 90%, and still more preferably at least 95% sequence identity to any one of the amino acid sequences set forth in SEQ ID NO: 87, 89, 91 or 93.

In yet another aspect, the present invention contemplates an isolated polypeptide encoding at least a portion of a methyltransferase (XabC) associated with albicidin biosynthesis or its variants or derivatives. In one embodiment of this type, the invention provides an isolated polypeptide comprising at least biologically active fragment or portion of the sequence set forth in SEQ ID NO: 95, or a variant or derivative thereof.

Suitably, the biologically active fragment comprises at least one, and preferably all, of the consensus methyltransferase sequence motifs set forth in SEQ ID NO: 99, 101 and 103, or variant thereof.

Preferably, the biologically active fragment comprises a contiguous sequence of amino acids contained within the sequence set forth in SEQ ID NO: 105, or variant thereof. In a preferred embodiment, the biologically active fragment comprises a contiguous

sequence of amino acids contained within the sequence set forth in SEQ ID NO: 107, or variant thereof.

Suitably, the variant has at least 60%, preferably at least 70%, more preferably at least 80%, more preferably at least 90% and still more preferably at least 95% sequence identity to the sequence set forth in SEQ ID NO: 95.

Preferably, the variant has at least 70%, preferably at least 80%, more preferably at least 90%, and still more preferably at least 95% sequence identity to any one of the amino acid sequences set forth in SEQ ID NO: 99, 101 and 103.

In still yet another aspect, the invention contemplates an isolated polynucleotide encoding at least a portion of an albicidin PKS-NRPS (XabB) or its variants or derivatives, as broadly described above. Preferably, the polynucleotide comprises the sequence set forth in any one of SEQ ID NO: 1 and 3, or a biologically active fragment thereof, or a polynucleotide variant of these.

Suitably, the biologically active fragment is at least 18 nucleotides in length.

The polynucleotide preferably encodes at least one domain selected from the group consisting of the AL domain, the KS domain, the KR domain, the ACP domain, the A domain, the PCP domain and the C domain as broadly described above.

Suitably, the AL domain is encoded by a nucleotide sequence set forth in any one or more of SEQ ID NO: 5 and 7, or variants thereof. Preferably, the AL domain is encoded by a nucleotide sequence comprising each of the sequences set forth in SEQ ID NO: 5 and 7, or variants thereof.

The KS domain is preferably encoded by a nucleotide sequence set forth in any one or more of SEQ ID NO: 9, 11, 13, 15, 17 and 19, or variants thereof. In one embodiment, the KS domain is preferably encoded by a nucleotide sequence comprising each of the sequences set forth in SEQ ID NO: 9, 11 and 13, or variants thereof. In an alternate embodiment, the KS domain is preferably encoded by a nucleotide sequence comprising each of the sequences set forth in SEQ ID NO: 15, 17 and 19, or variants thereof.

Preferably, the KR domain is encoded by a nucleotide sequence set forth in SEQ ID NO: 21, or variant thereof.

Suitably, the ACP domain is encoded by a nucleotide sequence set forth in any one or more of SEQ ID NO: 23, 25 and 27, or variants thereof.

The A domain is preferably encoded by a nucleotide sequence set forth in any one or more of SEQ ID NO: 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47, or variants thereof. In a preferred embodiment, the A domain is encoded by a nucleotide sequence comprising each of the sequences set forth in SEQ ID NO: 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47, or variants thereof.

Suitably, the PCP domain is encoded by a nucleotide sequence set forth in any one or more of SEQ ID NO: 49 and 51, or variants thereof.

Preferably, the C domain is encoded by a nucleotide sequence set forth in any one or more of SEQ ID NO: 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77 and 79, or variants thereof. In one embodiment, the C domain is preferably encoded by a nucleotide sequence comprising each of the sequences set forth in SEQ ID NO: 53, 55, 57, 59, 61, 63 and 65, or variants thereof. In an alternate embodiment, the C domain is preferably encoded by a nucleotide sequence comprising each of the sequences set forth in SEQ ID NO: 67, 69, 71, 73, 75, 77 and 79, or variants thereof.

In one embodiment, the polynucleotide variant has at least 60%, preferably at least 70%, more preferably at least 80%, and still more preferably at least 90% sequence identity to any one of the polynucleotides set forth in SEQ ID NO: 1 or 3.

In another embodiment, the polynucleotide variant is capable of hybridising to any one of the polynucleotides identified by SEQ ID NO: 1 or 3 under at least low stringency conditions, preferably under at least medium stringency conditions, and more preferably under high stringency conditions.

Preferably, the polynucleotide variant comprises a nucleotide sequence encoding at least one domain selected from the group consisting of the AL domain, the KS domain, the KR domain, the ACP domain, the A domain, the PCP domain and the C domain as broadly described above.

In one embodiment, the nucleotide sequence variant has at least 60%, preferably at least 70%, more preferably at least 80%, and still more preferably at least 90% sequence identity to any one of the sequences set forth in SEQ ID NO: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77 and 79.

In another embodiment, the nucleotide sequence variant is capable of hybridising to any one of the sequences identified by SEQ ID NO: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77 and 79 under at least low stringency conditions, preferably under at least medium stringency conditions, and more preferably under high stringency conditions.

In a further aspect, the invention contemplates an isolated polynucleotide encoding at least a portion of a PPTase (XabA) associated with albicidin biosynthesis or its variants or derivatives. an isolated polynucleotide encoding a polypeptide, fragment, variant or derivative as broadly described above. Preferably, the polynucleotide comprises the sequence set forth in any one of SEQ ID NO: 82 and 84, or a biologically active fragment thereof, or a polynucleotide variant of these.

Alternatively, the polynucleotide comprises a contiguous sequence of nucleotides contained within the sequence set forth in SEQ ID NO: 86, or variant thereof.

In one embodiment, the polynucleotide variant has at least 60%, preferably at least 70%, more preferably at least 80%, and still more preferably at least 90% sequence identity to any one of the polynucleotides set forth in SEQ ID NO: 82, 84 and 86.

In another embodiment, the polynucleotide variant is capable of hybridising to any one of the polynucleotides identified by SEQ ID NO: 82, 84 and 86 under at least low stringency conditions, preferably under at least medium stringency conditions, and more preferably under high stringency conditions.

Preferably, the polynucleotide variant comprises a nucleotide sequence encoding at least one PPTase sequence motif selected from SEQ ID NO: 89 and 93, or variant thereof. Suitably, the polynucleotide variant comprises a nucleotide sequence encoding the

intervening sequence between the said consensus PPTase sequence motifs, said nucleotide sequence comprising the sequence set forth in SEQ ID NO: 91.

The polynucleotide variant suitably comprises a nucleotide sequence encoding a contiguous sequence of amino acids contained within the sequence set forth in SEQ ID NO: 87, or variant thereof. In this instance, the contiguous sequence is preferably encoded by the sequence set forth in SEQ ID NO: 86, or nucleotide sequence variant thereof

Suitably, the PPTase sequence motif is encoded by a nucleotide sequence comprising the sequence set forth in any one of SEQ ID NO: 88 and 92, or nucleotide sequence variant thereof.

Preferably, the said intervening sequence is encoded by the nucleotide sequence set forth in SEQ ID NO: 90, or nucleotide sequence variant thereof.

In one embodiment, the nucleotide sequence variant has at least 60%, preferably at least 70%, more preferably at least 80%, and still more preferably at least 90% sequence identity to any one of the sequences set forth in SEQ ID NO: 86, 88, 90 and 92.

In another embodiment, the nucleotide sequence variant is capable of hybridising to any one of the sequences identified by SEQ ID NO: 86, 88, 90 and 92 under at least low stringency conditions, preferably under at least medium stringency conditions, and more preferably under high stringency conditions.

In yet a further aspect, the invention contemplates an isolated polynucleotide encoding at least a portion of a methyltransferase (XabC) associated with albicidin biosynthesis or its variants or derivatives. Preferably, the polynucleotide comprises the sequence set forth in any one of SEQ ID NO: 94 and 96, or a biologically active fragment thereof, or a polynucleotide variant of these.

Alternatively the polynucleotide comprises a contiguous sequence of nucleotides contained within the sequence set forth in SEQ ID NO: 104, or variant thereof. In one embodiment, this polynucleotide preferably comprises a contiguous sequence of nucleotides contained within the sequence set forth in SEQ ID NO: 106, or variant thereof

In one embodiment, the polynucleotide variant has at least 60%, preferably at least 70%, more preferably at least 80%, and still more preferably at least 90% sequence identity to any one of the polynucleotides set forth in SEQ ID NO: 94, 96, 104 and 106.

In another embodiment, the polynucleotide variant is capable of hybridising to any one of the polynucleotides identified by SEQ ID NO: 94, 96, 104 and 106 under at least low stringency conditions, preferably under at least medium stringency conditions, and more preferably under high stringency conditions.

Preferably, the polynucleotide variant comprises a nucleotide sequence encoding a methyltransferase sequence motif selected from any one or more of SEQ ID NO: 99, 101 and 103, or variant thereof.

Suitably, the methyltransferase sequence motif is encoded by a nucleotide sequence comprising the sequence set forth in any one of SEQ ID NO: 98, 100 and 102, or nucleotide sequence variant thereof.

In one embodiment, the nucleotide sequence variant has at least 60%, preferably at least 70%, more preferably at least 80%, and still more preferably at least 90% sequence identity to any one of the sequences set forth in SEQ ID NO: 98, 100 and 102.

In another embodiment, the nucleotide sequence variant is capable of hybridising to any one of the sequences identified by SEQ ID NO: 98, 100 and 102 under at least low stringency conditions, preferably under at least medium stringency conditions, and more preferably under high stringency conditions.

In still a further aspect, the invention features an expression vector comprising a polynucleotide as broadly described above wherein the polynucleotide is operably linked to a regulatory polynucleotide.

In another aspect, the invention provides a host cell containing a said expression vector.

Suitably, the host cell is a bacterium or other prokaryote.

In yet another aspect, the invention is directed to a multiplicity of cell colonies, constituting a library of colonies, wherein each colony of the library contains an expression

vector for the production of a polypeptide, fragment, variant or derivative as broadly described above.

The invention also features a method of producing a recombinant polypeptide, fragment, variant or derivative as broadly described above, comprising:

- culturing a host cell containing an expression vector as broadly described above such that said recombinant polypeptide, fragment, variant or derivative is expressed from said polynucleotide; and
- isolating the said recombinant polypeptide, fragment, variant or derivative.

In another aspect, the invention provides a method of producing a biologically active fragment of a polypeptide as broadly described above, comprising:

- detecting an activity associated with a fragment of the polypeptide set forth in SEQ ID NO: 2, wherein said activity is selected form the group consisting of acyl-CoA ligase activity, β-ketoacyl synthase activity, β-ketoacyl reductase, acyl carrier protein activity, adenylation activity, peptidyl carrier protein activity and condensation activity; or
- detecting PPTase activity associated with a fragment of the polypeptide set forth in SEQ ID NO: 83; or
- detecting methyltransferase activity associated with a fragment of the polypeptide set forth in SEQ ID NO: 95;

wherein detection of said activity is indicative of said fragment being a biologically active fragment.

In a further aspect, the invention provides a method of producing a biologically active fragment as broadly described above, comprising:

- introducing a polynucleotide from which a fragment of a polypeptide as broadly described above can be produced into a cell; and
- detecting an activity selected form the group consisting of acyl-CoA ligase activity, β-ketoacyl synthase activity, β-ketoacyl reductase, acyl carrier protein activity, adenylation activity, peptidyl carrier protein activity and condensation activity; or
- detecting PPTase activity associated with a fragment of the polypeptide set forth in SEQ ID NO: 83; or

– detecting methyltransferase activity associated with a fragment of the polypeptide set forth in SEQ ID NO: 95;

wherein detection of said activity is indicative of said fragment being a biologically active fragment.

In yet a further aspect, the invention provides a method of producing a variant of a polypeptide as broadly described above (parent polypeptide), or a biologically active fragment thereof, comprising:

– producing a modified polypeptide whose sequence is distinguished from the parent polypeptide or the biologically active fragment by substitution, deletion or addition of at least one amino acid; and

– detecting an activity associated with the modified polypeptide, wherein said activity is selected form the group consisting of acyl-CoA ligase activity, β-ketoacyl synthase activity, β-ketoacyl reductase, acyl carrier protein activity, adenylation activity, peptidyl carrier protein activity, condensation activity, PPTase activity and methyltransferase activity, wherein detection of said activity is indicative of said modified polypeptide being a variant.

In a further aspect, the invention contemplates a method of producing a variant of a parent polypeptide as broadly described above, or biologically active fragment thereof, comprising:

– producing a polynucleotide from which a modified polypeptide as described above can be produced;

– introducing said polynucleotide into a cell; and

– detecting an activity associated with the modified polypeptide, wherein said activity is selected form the group consisting of acyl-CoA ligase activity, β-ketoacyl synthase activity, β-ketoacyl reductase, acyl carrier protein activity, adenylation activity, peptidyl carrier protein activity, condensation activity, PPTase activity and methyltransferase activity, wherein detection of said activity is indicative of said modified polypeptide being a variant..

In yet another aspect, the invention extends to a method of screening for an agent that modulates the expression of a gene or variant thereof or the level and/or functional activity of an expression product of said gene or variant thereof, wherein said gene is

selected from *xabB*, *xabA*, or *xabC*, or a gene belonging to the same regulatory or biosynthetic pathway as *xabB*, *xabA*, or *xabC*, said method comprising:

– contacting a preparation comprising a polypeptide encoded by said gene, or biologically active fragment of said polypeptide, or variant or derivative of these, or a genetic sequence (*e.g.*, a transcriptional control element) that modulates the expression of said gene or variant thereof, with a test agent; and

– detecting a change in the level and/or functional activity of said polypeptide or biologically active fragment thereof, or variant or derivative, or of a product expressed from said genetic sequence.

The transcriptional control element preferably comprises the sequence set forth in SEQ ID NO: 81 or complement thereof.

The invention, in another aspect, also provides a method for enhancing the level and/or functional activity of an albicidin, said method comprising:

– introducing into an albicidin-producing host cell (1) an agent that modulates the expression of a gene encoding at least a portion of an albicidin PKS-NRPS or variant or derivative thereof, or the level and/or functional activity of an expression product of said gene, or (2) a vector from which a polynucleotide encoding at least a portion of an albicidin PKS-NRPS or variant or derivative thereof can be translated;

– and culturing the host cell for a time and under conditions sufficient to enhance the level and/or functional activity of said albicidin.

Preferably, the method further comprises introducing into said host cell a vector from which a PPTase can be translated. Suitably, the PPTase is selected from EntD or XabA.

Preferably, the method further comprises introducing into said host cell a vector from which a methyltransferase, more preferably and *O*-methyltransferase, and even more preferably an *S*-adenosylmethionine *O*-methyltransferase can be translated.

According to another aspect of the invention, there is provided a method for enhancing the level and/or functional activity of an albicidin, said method comprising contacting a precursor of said albicidin or an intermediate involved in the biosynthesis of said albicidin with at least a portion of an albicidin PKS-NRPS, or variant or derivative

thereof, as broadly described above, for a time and under conditions sufficient to enhance the level and/or functional activity of said albicidin.

Preferably, the method further comprises contacting a precursor of said albicidin or an intermediate involved in the biosynthesis of said albicidin with a PPTase.

Preferably, the method further comprises contacting a precursor of said albicidin or an intermediate involved in the biosynthesis of said albicidin with a methyltransferase, more preferably and *O*-methyltransferase, and even more preferably an *S*-adenosylmethionine *O*-methyltransferase.

In another aspect, the invention provides a method of identifying a PPTase for enhancing the level and/or functional activity of an albicidin, said method comprising introducing into an albicidin-deficient strain of *X. albilineans* which lacks *xabA* a vector comprising a polynucleotide encoding a test PPTase, wherein said polynucleotide is operably linked to a regulatory polynucleotide, and detecting production of albicidin.

Suitably, the strain is LS156 described herein.

Preferably, the PPTase is EntD.

The invention, in another aspect, also provides a method for enhancing the level and/or functional activity of an albicidin, said method comprising:

- introducing into an albicidin-producing host cell (1) an agent that modulates the expression of a gene encoding at least a portion of a PPTase associated with albicidin biosynthesis or variant or derivative thereof, or the level and/or functional activity of an expression product of said gene, or (2) a vector from which a polynucleotide encoding at least a portion of a PPTase associated with albicidin biosynthesis or variant or derivative thereof can be translated;
- and culturing the host cell for a time and under conditions sufficient to enhance the level and/or functional activity of said albicidin

In yet another aspect, the invention provides a method for enhancing the level and/or functional activity of an albicidin, said method comprising:

- introducing into an albicidin-producing host cell (1) an agent that modulates the expression of a gene encoding at least a portion of a methyltransferase associated

with albicidin biosynthesis or variant or derivative thereof, or the level and/or functional activity of an expression product of said gene, or (2) a vector from which a polynucleotide encoding at least a portion of a methyltransferase associated with albicidin biosynthesis or variant or derivative thereof can be translated;

– and culturing the host cell for a time and under conditions sufficient to enhance the level and/or functional activity of said albicidin

In another aspect, the invention resides in an antigen-binding molecule that is immuno-interactive with a polypeptide, fragment, variant or derivative as broadly described above.

In yet another aspect, the invention provides a method to prepare a polynucleotide encoding a modified PKS, comprising using an albicidin PKS-NRPS encoding nucleotide sequence as a scaffold and modifying the portions of the nucleotide sequence that encode enzymatic activities, either by mutagenesis, inactivation, deletion, insertion, or replacement.

In still yet another aspect, the invention contemplates a method for producing polyketides, comprising expressing the modified albicidin PKS encoding nucleotide sequence as broadly described in a suitable host cell to thereby produce a polyketide different from that produced by the albicidin PKS-NRPS.

Another aspect of the invention contemplates the insertion of portions of the albicidin PKS-NRPS coding sequence into other PKS coding sequences to modify the products thereof.

In a further aspect, the invention encompasses use of the polypeptide, fragment, variant or derivative as broadly described above, or the polynucleotide or vector as broadly described above, or the modulatory agent as broadly described above for producing secondary metabolites, preferably albicidins.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation showing a physical and functional map of part of the albicidin biosynthetic gene cluster. (A). Partial physical map of the Tn5 insertion locus in LS157 genomic DNA. Restriction enzymes used: C, *Cla*I; E, *Eco*RI; S, *Spe*I; N, *Not*I; and B, *Bam*HI. (B). Probes used to recover clone pXABB: Probe 1, 1.4-kb *Eco*RI-*Not*I fragment digested from pBC157; and probe 2, 0.9-kb PCR product amplified from Xa13 genomic DNA using primers complementary to sequences flanking the Tn5 insertion in LS157. (C). Clones and subclones used for sequencing, and described in Table 1. (D). The transcription directions of three putative ORFs in 16.5-kb *Eco*RI fragment are indicated by arrows. (E). Organisation of *X. albilineans* XabB constructed by comparison with known protein sequences. The unshaded box indicates PKS region, and the shade box indicates NRPS region. Relative positions of potential catalytic domains or active sites are indicated by: AL, acyl-CoA ligase; ACP, acyl carrier protein; KS, β-ketoacyl synthase; KR, β-ketoacyl reductase; PCP, peptidyl carrier protein; C, condensation; A, adenylation. Horizontal bars indicate proposed biosynthetic modules.

Figure 2 is a diagrammatic representation presenting the sequence of the region upstream from *xabB*. The nucleotide sequence is numbered according to the 16511-bp sequence in GenBank accession no. AF239749. The putative –35 and –10 promoter sequences of *xabB* and the divergent gene *xatA* are underlined, as are ribosome-binding sequences. The transcriptional directions of *xabB* and *xatA* are indicated by arrows. Translational start codons are indicated by boldface type. Primers P1F1 and P1R are shaded.

Figure 3 is a diagrammatic representation showing the alignment of *X. albilineans* XabB enzymatic domains with those of PKSs and FASs from other organisms. Identical amino acids are indicated by boldface type. Stars and overlines identify conserved amino acids at catalytic sites. Xal-XabB, *X. albilineans* XabB for biosynthesis of albicidin (this study); Hin-LCFA, *Haemophilus influenza* long-chain fatty acid-CoA ligase (P46450); Bsu-PksJ, *B. subtilis* polyketide synthase J (P40806); Bsu-MycA, *B. subtilis* MycA for biosynthesis of mycosubtilin (AF184956); Pcr-ComL2, *Petroselinum crispum* 4-coumarate-CoA ligase 2 (P14913); Sma-FkbB, *S.* sp. MA6548 FkbB for biosynthesis of FK506 (AF082099); Ame-RifA, *Amycolatopsis mediterranei* RifA for biosynthesis of

rifamycin B (AF040570); Shy-RapA, *S. hygroscopicus* RapA for biosynthesis of rapamycin (X86780); Mxa-Ta1, *M. xanthus* Ta1 for biosynthesis of TA (AJ006977); Ser-EryA1 and EryA3, *S. erythraea* EryA modules for biosynthesis of erythromycin (M63676, M63677); Che-PKS1, *Cochliobolus heterostrophus* PKS1 for biosynthesis of T-toxin (U68040); Bsu-PksM, *B. subtilis* PKS for a polyketide synthase (O31781); Mtu-PpsA, *M. tuberculosis* PKS for a polyketide synthase (G3261605); Mtu-MAS, *M. tuberculosis* MAS for biosynthesis of mycocerosic acid (M95808); Chick-FAS, chichen fatty acid synthase (M22987); Rat-FAS, rat fatty acid synthase (X14175).

Figure 4 is a graphical representation showing albicidin production by wild-type *X. albilineans* LS155 (▲), complemented Tox⁻ mutant strain LS157 pLXABB1 (O), complemented Tox⁻ mutant strain LS157 pLXABB2 (●), LS157 (■), and LS157 pLAFR3 (+). Albicidin concentrations in culture supernatants were quantified based on inhibition zone width in a microbial bioassay (means +/- standard errors from 5 replicates).

Figure 5 is a graphical representation showing the relationship between growth (■), albicidin production (O), and GUS activity (▲) in *X. albilineans* LS155 pRG960p1 (A) and in LS155 pRG960p2 (B). Relative activity (means +/- standard errors from 2 replicates): 100% growth, OD_{550} = 1.43; 100% albicidin production = 268.5 units/ml; 100% GUS activity = 119 units/mg of protein (one unit equals 1 pmol of methylumbelliferone formed per min.). Locations and sizes of inserts on pRG960p1 and pRG960p2 are indicated in Figure 2 and Table 1. GUS, β-glucuronidase.

Figure 6 is a schematic representation showing the organisation of five known PKS-NRPS enzymes. *X. albilineans* XabB, encoded by *xabB* for albicidin biosynthesis (this study); *B. subtilis* MycA for mycosubtilin biosynthesis (Duitman *et al.*, 1999); *Yersinia pestis* HMWP1 for yersiniabactin biosynthesis (Gehring *et al.*, 1998); *M. xanthus* partial gene product Ta1 for TA biosynthesis (Paitan *et al.*, 1999); *B. subtilis* PksorfX6 for unknown function (Albertini *et al.*, 1995). Unshaded boxes indicate PKS regions, grey boxes indicate NRPS regions, and dark boxes indicate amino transferase (AMT) or methyltransferase (MT). Vertical bars follow the carrier domains at the end of each biosynthetic "module".

Figure 7 is a diagrammatic representation showing a dendrogram (GCG) analysis of adenylation domains of XabB and its homologous peptide synthetases. Peptide

synthetases, including various modules of the same multienzyme complex, are as follows: GrsA and GrsB, gramicidin synthetase A and B, respectively, from *B. subtilis* (X15577, X61658); BacA, BacB, and BacC, bacitracin synthetase A, B, and C, respectively, from *B. licheniformis* (AF007865); SnbC and SnbDE, pristinamycin I synthetase C and DE, respectively, from *S. pristinaespiralis* (X98690, Y11547); FkbP, FK506 synthetase FkbP from *S.* sp. MA6548 (AF082100); TycA, TycB, and TycC, tyrocidine synthetase A, B, and C, respectively, from *B. brevis* (AF004835); SyrE, syringomycin synthetase E1 from *Pseudomonas syringae* pv. *syringae* (AF047828); EntF, enterobactin synthetase F from *E. coli* (P11454); DhbF, 2,3-dihydroxybenzoate synthetase F from *B. subtilis* (P45745); FenD, fengycin synthetase FenD1 from *B. subtilis* (AJ011849); SrfAA, SrfAB, and SrfAC, surfactin A synthetase A, B, and C, respectively, from *B. subtilus* (X70356); XabB, albicidin synthase B from *X. albilineans* (this study). The A4 to A5 regions (about 100 aa) of adenylation domains of peptide synthetases, which is involved in amino acid recognition and binding, were aligned using the PILEUP program with default parameters.

Figure 8 is a diagrammatic representation showing a restriction map of clones including the *xabA* gene from *X. albilineans*. Sequencing by primer walking commenced at the T3 and T7 primers. The location and direction of transcription of the *xabA* ORF is shown by an arrow. Restriction enzymes are: E, *Eco*RI; P, *Pst*I; C, *Cla*I; and H, *Hin*dIII

Figure 9 is a diagrammatic representation presenting the sequence of the *xabA* gene. The nucleotide sequence is numbered according to the 3-kb sequence in GenBank accession no. AF191324. The closest matches to RBS region and promoter consensus sequences are underlined, as are the region of dyad symmetry and putative factor-independent termination sites. Translation start and stop codons are indicated by boldface type. The (V/I)G(V/I)D and (F/W)(S/C/T)xKE(A/S)xxK motifs conserved in PPTase enzymes are boxed. The insertion site of Tn5 is marked (▼).

Figure 10 is a graphical representation showing albicidin production by wild-type *X. albilineans* strain Xa13 (O), Xa13 pLXABA (•), and complemented Tox$^-$ mutant strain LS156 pLXABA (▲). Albicidin concentrations in culture supernatants were quantified based on inhibition zone width in a microbial bioassay (means +/- standard errors from 2 replicates).

Figure 11 is a schematic representation showing a dendrogram (GCG) analysis of PPTases involved in antibiotic and fatty acid biosynthesis in bacteria. Sau, *Salmonella austin*; Sty, *Salmonella typhymurium*; Bbr, *Bacillus brevis*; Xal, *Xanthomonas albilineans*; Eco, *Escherichia coli*; Sfl, *Shigella flexneri*; Bpu, *Bacillus pumilus*; Bsu, *Bacillus subtilis*; Mtu, *Mycobacterium tuberculosis*; Hin, *Haemophilus influenzae*. The sources of amino acid sequence of PPTases correspond to those in Table 2, and the sequences were aligned using the PILEUP program with default parameters.

Figure 12 is a schematic representation showing the organisation of part of the albicidin biosynthetic gene cluster. The location and direction of three ORFs are indicated by thick arrows. Vertical lines indicate the position of restriction enzyme sites: E, *Eco*RI; B, *Bam*HI; S, *Spe*I; N, *Nco*I. The vertical lines with triangles (▲) show the position of insertional mutagenesis sites or Tn5 insertion site, and the resultant mutants are bracketed. The arrows above the physical map indicate the locations of primers used to amplify sequence downstream of the *Eco*RI restriction site by IPCR. The cloned regions for complementation tests are shown below the map.

Figure 13 is a diagrammatic representation presenting the nucleotide and deduced amino acid sequences of the *xabC* region. The nucleotide sequence is numbered according to the 1515-bp sequence in GenBank accession no. AF239750. The potential RBS and selected restriction sites are underlined. The putative factor-independent termination signals are underlined and indicated by bold letters. Translation start and stop codons are indicated by bold letters. The conserved motifs in Mtases are boxed. Primers used for PCR (A3F and A3R) and IPCR (IR) are shaded.

Figure 14 is a diagrammatic representation showing the conserved sequence motifs in Mtases involved in antibiotic biosynthesis in bacteria. Identical or similar amino acids (A = G; D = E; I = L = V) are shown in bold. Numbers indicate amino acid residues from the N terminus of the protein. Xal-XabC, putative albicidin biosynthesis Mtase from *X. albilineans* (this study); Sgl-TcmO and Sgl-TcmN, multifunctional cyclase-dehydrase-3-*O*-Mtase and tetracenomycin polyketide synthesis 8-*O*-Mtase of *Streptomyces glaucescens*, respectively (accession number M80674); Smy-MdmC, midecamycin-*O*-Mtase of *S. mycarofaciens* (M93958); Mxa-SafC, saframycin *O*-Mtase of *Myxococcus xanthus* (U24657); Ser-EryG, erythromycin biosynthesis *O*-Mtase of *Saccharopolyspora*

erythraea (S18533); Spe-DauK, carminomycin 4-*O*-Mtase from *S. peucetius* (L13453); Sal-DmpM, *O*-demethylpuromycin-*O*-Mtase from *S. alboniger* (M74560); Shy-RapM, rapamycin *O*-Mtase of *S. hygroscopicus* (X86780); Sav-AveD, avermectin B 5-*O*-Mtase from *S. avermitilis* (G5921167).

Figure 15 is a graphical representation showing albicidin production by wild-type *X. albilineans* LS155 (•), Tox$^-$ *xabC* insertion mutant LS-JP2 (■), complemented strain LS-JP2 pLXABC containing Lac promoter – full length *xabC* gene (O), and complemented strain LS-JP2 pLXABB1 containing full length *xabB* plus functional N-terminal region of *xabC* (∏). Albicidin concentrations in culture supernatants were quantified based on inhibition zone width in a microbial bioassay (means +/- standard errors from 2 or 3 replicates).

BRIEF DESCRIPTION OF THE SEQUENCES: SUMMARY TABLE

TABLE A

SEQUENCE ID NUMBER	SEQUENCE	LENGTH
SEQ ID NO: 1	Full-length *xabB* (Accession No. AF239749)	16551 bases
SEQ ID NO: 2	Full-length polypeptide sequence encoded by SEQ ID NO: 1	4801 residues
SEQ ID NO: 3	Full-length coding sequence of *xabB*	14406 bases
SEQ ID NO: 4	Polypeptide sequence encoded by SEQ ID NO: 3	4801 residues
SEQ ID NO: 5	Sub-sequence of SEQ ID NO: 1 and 3 encoding acyl-CoA ligase subdomain I	45 bases
SEQ ID NO: 6	Acyl-CoA ligase subdomain I encoded by SEQ ID NO: 5	15 residues
SEQ ID NO: 7	Sub-sequence of SEQ ID NO: 1 and 3 encoding acyl-CoA ligase subdomain II	24 bases
SEQ ID NO: 8	Acyl-CoA ligase subdomain I encoded by SEQ ID NO: 7	8 residues
SEQ ID NO: 9	Sub-sequence of SEQ ID NO: 1 and 3 encoding β-ketoacyl synthase 1 subdomain I	51 bases
SEQ ID NO: 10	β-Ketoacyl synthase 1 subdomain I encoded by SEQ ID NO: 9	17 residues
SEQ ID NO: 11	Sub-sequence of SEQ ID NO: 1 and 3 encoding β-ketoacyl synthase 1 subdomain II	30 bases
SEQ ID NO: 12	β-Ketoacyl synthase 1 subdomain II encoded by SEQ ID NO: 11	10 residues
SEQ ID NO: 13	Sub-sequence of SEQ ID NO: 1 and 3 encoding β-ketoacyl synthase 1 subdomain III	30 bases
SEQ ID NO: 14	β-Ketoacyl synthase 1 subdomain III encoded by SEQ ID NO: 13	10 residues
SEQ ID NO: 15	Sub-sequence of SEQ ID NO: 1 and 3 encoding β-ketoacyl synthase 2 subdomain I	51 bases

SEQUENCE ID NUMBER	SEQUENCE	LENGTH
SEQ ID NO: 16	β-Ketoacyl synthase 2 subdomain I encoded by SEQ ID NO: 15	17 residues
SEQ ID NO: 17	Sub-sequence of SEQ ID NO: 1 and 3 encoding β-ketoacyl synthase 2 subdomain II	30 bases
SEQ ID NO: 18	β-Ketoacyl synthase 2 subdomain II encoded by SEQ ID NO: 17	10 residues
SEQ ID NO: 19	Sub-sequence of SEQ ID NO: 1 and 3 encoding β-ketoacyl synthase 2 subdomain III	30 bases
SEQ ID NO: 20	β-Ketoacyl synthase 2 subdomain III encoded by SEQ ID NO: 19	10 residues
SEQ ID NO: 21	Sub-sequence of SEQ ID NO: 1 and 3 encoding β-ketoacyl reductase domain	93 bases
SEQ ID NO: 22	β-Ketoacyl reductase domain encoded by SEQ ID NO: 21	31 residues
SEQ ID NO: 23	Sub-sequence of SEQ ID NO: 1 and 3 encoding acyl carrier protein 1 domain	36 bases
SEQ ID NO: 24	Acyl carrier protein 1 domain encoded by SEQ ID NO: 23	12 residues
SEQ ID NO: 25	Sub-sequence of SEQ ID NO: 1 and 3 encoding acyl carrier protein 2 domain	36 bases
SEQ ID NO: 26	Acyl carrier protein 2 domain encoded by SEQ ID NO: 25	12 residues
SEQ ID NO: 27	Sub-sequence of SEQ ID NO: 1 and 3 encoding acyl carrier protein 3 domain	36 bases
SEQ ID NO: 28	Acyl carrier protein 3 domain encoded by SEQ ID NO: 27	12 residues
SEQ ID NO: 29	Sub-sequence of SEQ ID NO: 1 and 3 encoding adenylation domain subdomain I	18 bases
SEQ ID NO: 30	Adenylation domain subdomain I encoded by SEQ ID NO: 29	6 residues
SEQ ID NO: 31	Sub-sequence of SEQ ID NO: 1 and 3 encoding adenylation domain subdomain II	33 bases

SEQUENCE ID NUMBER	SEQUENCE	LENGTH
SEQ ID NO: 32	Adenylation domain subdomain II encoded by SEQ ID NO: 31	11 residues
SEQ ID NO: 33	Sub-sequence of SEQ ID NO: 1 and 3 encoding adenylation domain subdomain III	48 bases
SEQ ID NO: 34	Adenylation domain subdomain III encoded by SEQ ID NO: 33	16 residues
SEQ ID NO: 35	Sub-sequence of SEQ ID NO: 1 and 3 encoding adenylation domain subdomain IV	12 bases
SEQ ID NO: 36	Adenylation domain subdomain IV encoded by SEQ ID NO: 35	4 residues
SEQ ID NO: 37	Sub-sequence of SEQ ID NO: 1 and 3 encoding adenylation domain subdomain V	21 bases
SEQ ID NO: 38	Adenylation domain subdomain V encoded by SEQ ID NO: 37	7 residues
SEQ ID NO: 39	Sub-sequence of SEQ ID NO: 1 and 3 encoding adenylation domain subdomain VI	45 bases
SEQ ID NO: 40	Adenylation domain subdomain VI encoded by SEQ ID NO: 39	15 residues
SEQ ID NO: 41	Sub-sequence of SEQ ID NO: 1 and 3 encoding adenylation domain subdomain VII	18 bases
SEQ ID NO: 42	Adenylation domain subdomain VII encoded by SEQ ID NO: 41	6 residues
SEQ ID NO: 43	Sub-sequence of SEQ ID NO: 1 and 3 encoding adenylation domain subdomain VIII	60 bases
SEQ ID NO: 44	Adenylation domain subdomain VIII encoded by SEQ ID NO: 43	20 residues
SEQ ID NO: 45	Sub-sequence of SEQ ID NO: 1 and 3 encoding adenylation domain subdomain IX	21 bases
SEQ ID NO: 46	Adenylation domain subdomain IX encoded by SEQ ID NO: 45	7 residues
SEQ ID NO: 47	Sub-sequence of SEQ ID NO: 1 and 3 encoding adenylation domain subdomain X	18 bases

SEQUENCE ID NUMBER	SEQUENCE	LENGTH
SEQ ID NO: 48	Adenylation domain subdomain X encoded by SEQ ID NO: 47	6 residues
SEQ ID NO: 49	Sub-sequence of SEQ ID NO: 1 and 3 encoding peptidyl carrier protein 1 domain	33 bases
SEQ ID NO: 50	Peptidyl carrier protein 1 domain encoded by SEQ ID NO: 49	11 residues
SEQ ID NO: 51	Sub-sequence of SEQ ID NO: 1 and 3 encoding peptidyl carrier protein 2 domain	33 bases
SEQ ID NO: 52	Peptidyl carrier protein 2 domain encoded by SEQ ID NO: 51	11 residues
SEQ ID NO: 53	Sub-sequence of SEQ ID NO: 1 and 3 encoding condensation domain 1 subdomain I	30 bases
SEQ ID NO: 54	Condensation domain 1 subdomain I encoded by SEQ ID NO: 53	10 residues
SEQ ID NO: 55	Sub-sequence of SEQ ID NO: 1 and 3 encoding condensation domain 1 subdomain II	27 bases
SEQ ID NO: 56	Condensation domain 1 subdomain II encoded by SEQ ID NO: 55	9 residues
SEQ ID NO: 57	Sub-sequence of SEQ ID NO: 1 and 3 encoding condensation domain 1 subdomain III	30 bases
SEQ ID NO: 58	Condensation domain 1 subdomain III encoded by SEQ ID NO: 57	10 residues
SEQ ID NO: 59	Sub-sequence of SEQ ID NO: 1 and 3 encoding condensation domain 1 subdomain IV	21 bases
SEQ ID NO: 60	Condensation domain 1 subdomain IV encoded by SEQ ID NO: 59	7 residues
SEQ ID NO: 61	Sub-sequence of SEQ ID NO: 1 and 3 encoding condensation domain 1 subdomain V	36 bases
SEQ ID NO: 62	Condensation domain 1 subdomain V encoded by SEQ ID NO: 61	12 residues
SEQ ID NO: 63	Sub-sequence of SEQ ID NO: 1 and 3 encoding condensation domain 1 subdomain VI	21 bases

SEQUENCE ID NUMBER	SEQUENCE	LENGTH
SEQ ID NO: 64	Condensation domain 1 subdomain VI encoded by SEQ ID NO: 63	7 residues
SEQ ID NO: 65	Sub-sequence of SEQ ID NO: 1 and 3 encoding condensation domain 1 subdomain VII	24 bases
SEQ ID NO: 66	Condensation domain 1 subdomain VII encoded by SEQ ID NO: 65	8 residues
SEQ ID NO: 67	Sub-sequence of SEQ ID NO: 1 and 3 encoding condensation domain 2 subdomain I	30 bases
SEQ ID NO: 68	Condensation domain 2 subdomain I encoded by SEQ ID NO: 67	10 residues
SEQ ID NO: 69	Sub-sequence of SEQ ID NO: 1 and 3 encoding condensation domain 2 subdomain II	27 bases
SEQ ID NO: 70	Condensation domain 2 subdomain II encoded by SEQ ID NO: 69	9 residues
SEQ ID NO: 71	Sub-sequence of SEQ ID NO: 1 and 3 encoding condensation domain 2 subdomain III	30 bases
SEQ ID NO: 72	Condensation domain 2 subdomain III encoded by SEQ ID NO: 71	10 residues
SEQ ID NO: 73	Sub-sequence of SEQ ID NO: 1 and 3 encoding condensation domain 2 subdomain IV	21 bases
SEQ ID NO: 74	Condensation domain 2 subdomain IV encoded by SEQ ID NO: 73	7 residues
SEQ ID NO: 75	Sub-sequence of SEQ ID NO: 1 and 3 encoding condensation domain 2 subdomain V	33 bases
SEQ ID NO: 76	Condensation domain 2 subdomain V encoded by SEQ ID NO: 75	11 residues
SEQ ID NO: 77	Sub-sequence of SEQ ID NO: 1 and 3 encoding condensation domain 2 subdomain VI	21 bases
SEQ ID NO: 78	Condensation domain 2 subdomain VI encoded by SEQ ID NO: 77	7 residues
SEQ ID NO: 79	Sub-sequence of SEQ ID NO: 1 and 3 encoding condensation domain 2 subdomain VII	24 bases

SEQUENCE ID NUMBER	SEQUENCE	LENGTH
SEQ ID NO: 80	Condensation domain 2 subdomain VII encoded by SEQ ID NO: 79	8 residues
SEQ ID NO: 81	Polynucleotide comprising *xabB* promoter	242 bases
SEQ ID NO: 82	Full-length *xabA* (Accession No. AF191324)	1200 bases
SEQ ID NO: 83	Full-length polypeptide sequence encoded by SEQ ID NO: 82	278 residues
SEQ ID NO: 84	Full-length coding sequence of *xabA*	837 bases
SEQ ID NO: 85	Polypeptide sequence encoded by SEQ ID NO: 84	278 residues
SEQ ID NO: 86	Sub-sequence of SEQ ID NO: 82 encoding PPTase domain	168 bases
SEQ ID NO: 87	PPTase domain encoded by SEQ ID NO: 86	56 residues
SEQ ID NO: 88	Sub-sequence of SEQ ID NO: 82 encoding a motif (motif I) conserved in PPTases	27 bases
SEQ ID NO: 89	PPTase motif I amino acid sequence encoded by SEQ ID NO: 88	9 residues
SEQ ID NO: 90	Sub-sequence of SEQ ID NO: 82 encoding intervening amino acid sequence linking motifs I and II	117 bases
SEQ ID NO: 91	Intervening amino acid sequence encoded by SEQ ID NO: 90	39 residues
SEQ ID NO: 92	Sub-sequence of SEQ ID NO: 82 encoding a motif (motif II) conserved in PPTases	36 bases
SEQ ID NO: 93	PPTase motif II amino acid sequence encoded by SEQ ID NO: 92	12 residues
SEQ ID NO: 94	Full-length *xabC* (Accession No. AF239750)	1515 bases
SEQ ID NO: 95	Full-length polypeptide sequence encoded by SEQ ID NO: 94	343 residues
SEQ ID NO: 96	Full-length coding sequence of *xabC*	1029 bases
SEQ ID NO: 97	Polypeptide sequence encoded by SEQ ID NO: 96	343 residues

SEQUENCE ID NUMBER	SEQUENCE	LENGTH
SEQ ID NO: 98	Sub-sequence of SEQ ID NO: 94 encoding a motif (motif I) conserved in methyltransferases	21 bases
SEQ ID NO: 99	Methyltransferase motif I amino acid sequence encoded by SEQ ID NO: 98	7 residues
SEQ ID NO: 100	Sub-sequence of SEQ ID NO: 94 encoding a motif (motif II) conserved in methyltransferases	24 bases
SEQ ID NO: 101	Methyltransferase motif II amino acid sequence encoded by SEQ ID NO: 100	8 residues
SEQ ID NO: 102	Sub-sequence of SEQ ID NO: 94 encoding a motif (motif III) conserved in methyltransferases	27 bases
SEQ ID NO: 103	Methyltransferase motif III amino acid sequence encoded by SEQ ID NO: 102	9 residues
SEQ ID NO: 104	Polynucleotide encoding said motifs I, II and III	303 bases
SEQ ID NO: 105	Polypeptide encoded by SEQ ID NO: 104	101 residues
SEQ ID NO: 106	Biologically active fragment of SEQ ID NO: 94	831 bases
SEQ ID NO: 107	Biologically active fragment of SEQ ID NO: 95	277 residues

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

The articles *"a"* and *"an"* are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

The term *"about"* is used herein to refer to sequences that vary by as much as 30%, preferably by as much as 20% and more preferably by as much as 10% to the length of a reference sequence.

By *"agent"* is meant a naturally occurring or synthetically produced molecule which interacts either directly or indirectly with a target member, the level and/or functional activity of which are to be modulated.

"Amplification product" refers to a nucleic acid product generated by nucleic acid amplification techniques.

By *"antigen-binding molecule"* is meant a molecule that has binding affinity for a target antigen. It will be understood that this term extends to immunoglobulins, immunoglobulin fragments and non-immunoglobulin derived protein frameworks that exhibit antigen-binding activity.

As used herein, the term *"binds specifically"* and the like refers to antigen-binding molecules that bind the polypeptide or polypeptide fragments of the invention but do not significantly bind to homologous prior art polypeptides.

By *"biologically active fragment"* is meant a fragment of a full-length parent polypeptide which fragment retains the activity of the parent polypeptide. A biologically active fragment will therefore comprise an activity selected form the group consisting of acyl-CoA ligase activity, β-ketoacyl synthase activity, β-ketoacyl reductase, acyl carrier protein activity, adenylation activity, peptidyl carrier protein activity, condensation activity, PPTase activity and methyltransferase activity. As used herein, the term *"biologically active fragment"* includes deletion mutants and small peptides, for example of at least 10, preferably at least 20 and more preferably at least 30 contiguous amino acids, which comprise the above activities. Peptides of this type may be obtained through the application of standard recombinant nucleic acid techniques or synthesised using conventional liquid or solid phase synthesis techniques. For example, reference may be made to solution synthesis or solid phase synthesis as described, for example, in Chapter 9 entitled *"Peptide Synthesis"* by Atherton and Shephard which is included in a publication entitled *"Synthetic Vaccines"* edited by Nicholson and published by Blackwell Scientific Publications. Alternatively, peptides can be produced by digestion of a polypeptide of the invention with proteinases such as endoLys-C, endoArg-C, endoGlu-C and staphylococcus V8-protease. The digested fragments can be purified by, for example, high performance liquid chromatographic (HPLC) techniques.

Throughout this specification, unless the context requires otherwise, the words *"comprise"*, *"comprises"* and *"comprising"* will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

By "*corresponds to*" or "*corresponding to*" is meant a polynucleotide (a) having a nucleotide sequence that is substantially identical or complementary to all or a portion of a reference polynucleotide sequence or (b) encoding an amino acid sequence identical to an amino acid sequence in a peptide or protein. This phrase also includes within its scope a peptide or polypeptide having an amino acid sequence that is substantially identical to a sequence of amino acids in a reference peptide or protein.

By *"derivative"* is meant a polypeptide that has been derived from the basic sequence by modification, for example by conjugation or complexing with other chemical moieties or by post-translational modification techniques as would be understood in the art.

The term "*derivative*" also includes within its scope alterations that have been made to a parent sequence including additions, or deletions that provide for functionally equivalent molecules. Accordingly, the term derivative encompasses molecules that will have an activity selected form the group consisting of acyl-CoA ligase activity, β-ketoacyl synthase activity, β-ketoacyl reductase, acyl carrier protein activity, adenylation activity, peptidyl carrier protein activity, condensation activity, PPTase activity and methyltransferase activity.

"Homology" refers to the percentage number of amino acids that are identical or constitute conservative substitutions as defined in Table B *infra*. Homology may be determined using sequence comparison programs such as GAP (Deveraux *et al.* 1984, *Nucleic Acids Research* **12**, 387-395). In this way, sequences of a similar or substantially different length to those cited herein might be compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by GAP.

"*Hybridisation*" is used herein to denote the pairing of complementary nucleotide sequences to produce a DNA-DNA hybrid or a DNA-RNA hybrid. Complementary base sequences are those sequences that are related by the base-pairing rules. In DNA, A pairs with T and C pairs with G. In RNA U pairs with A and C pairs with G. In this regard, the terms "match" and "mismatch" as used herein refer to the hybridisation potential of paired nucleotides in complementary nucleic acid strands. Matched nucleotides hybridise efficiently, such as the classical A-T and G-C base pair mentioned above. Mismatches are other combinations of nucleotides that do not hybridise efficiently.

Reference herein to *"immuno-interactive"* includes reference to any interaction, reaction, or other form of association between molecules and in particular where one of the molecules is, or mimics, a component of the immune system.

By *"immuno-interactive fragment"* is meant a fragment of a parent or reference polypeptide as described herein, which fragment elicits an immune response, including the production of elements that specifically bind to said polypeptide, or variant or derivative thereof. As used herein, the term *"immuno-interactive fragment"* includes deletion mutants and small peptides, for example of at least six, preferably at least 8 and more preferably at

least 20 contiguous amino acids, which comprise antigenic determinants or epitopes. Several such fragments may be joined together.

By *"isolated"* is meant material that is substantially or essentially free from components that normally accompany it in its native state. For example, an "isolated polynucleotide", as used herein, refers to a polynucleotide, which has been purified from the sequences which flank it in a naturally occurring state, *e.g.*, a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment.

By *"modulating"* is meant increasing or decreasing, either directly or indirectly, the level and/or functional activity of a target molecule. For example, an agent may indirectly modulate the said level/activity by interacting with a molecule other than the target molecule. In this regard, indirect modulation of a gene encoding a target polypeptide includes within its scope modulation of the expression of a first nucleic acid molecule, wherein an expression product of the first nucleic acid molecule modulates the expression of a nucleic acid molecule encoding the target polypeptide.

By *"obtained from"* is meant that a sample such as, for example, a nucleic acid extract or polypeptide extract is isolated from, or derived from, a particular source. For example, the extract may be isolated directly from any organism that produces secondary metabolites, preferably from an albicidin-producing microorganism, more preferably from microorganisms of the genus *Xanthomonas*.

The term "*oligonucleotide*" as used herein refers to a polymer composed of a multiplicity of nucleotide units (deoxyribonucleotides or ribonucleotides, or related structural variants or synthetic analogues thereof) linked via phosphodiester bonds (or related structural variants or synthetic analogues thereof). Thus, while the term "oligonucleotide" typically refers to a nucleotide polymer in which the nucleotides and linkages between them are naturally occurring, it will be understood that the term also includes within its scope various analogues including, but not restricted to, peptide nucleic acids (PNAs), phosphoramidates, phosphorothioates, methyl phosphonates, 2-O-methyl ribonucleic acids, and the like. The exact size of the molecule may vary depending on the particular application. An oligonucleotide is typically rather short in length, generally from about 10 to 30 nucleotides, but the term can refer to molecules of any length, although the term "polynucleotide" or "nucleic acid" is typically used for large oligonucleotides.

By *"operably linked"* is meant that transcriptional and translational regulatory nucleic acids are positioned relative to a polypeptide-encoding polynucleotide in such a manner that the polynucleotide is transcribed and the polypeptide is translated.

The term "*polynucleotide*" or "*nucleic acid*" as used herein designates mRNA, RNA, cRNA, cDNA or DNA. The term typically refers to oligonucleotides greater than 30 nucleotides in length.

The terms *"polynucleotide variant"* and *"variant"* refer to polynucleotides displaying substantial sequence identity with a reference polynucleotide sequence or polynucleotides that hybridise with a reference sequence under stringent conditions that are defined hereinafter. These terms also encompass polynucleotides in which one or more nucleotides have been added or deleted, or replaced with different nucleotides. In this regard, it is well understood in the art that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference polynucleotide whereby the altered polynucleotide retains the biological function or activity of the reference polynucleotide. The terms *"polynucleotide variant"* and *"variant"* also include naturally occurring allelic variants.

"*Polypeptide*", "*peptide*" and "*protein*" are used interchangeably herein to refer to a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues is a synthetic non-naturally occurring amino acid, such as a chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally-occurring amino acid polymers.

The term *"polypeptide variant"* refers to polypeptides in which one or more amino acids have been replaced by different amino acids. It is well understood in the art that some amino acids may be changed to others with broadly similar properties without changing the nature of the activity of the polypeptide (conservative substitutions) as described hereinafter. These terms also encompass polypeptides in which one or more amino acids have been added or deleted, or replaced with different amino acids. Accordingly, polypeptide variants as used herein encompass polypeptides that have an activity selected form the group consisting of acyl-CoA ligase activity, β-ketoacyl synthase activity, β-ketoacyl reductase, acyl carrier protein activity, adenylation activity, peptidyl

carrier protein activity, condensation activity, PPTase activity and methyltransferase activity.

By "*primer*" is meant an oligonucleotide which, when paired with a strand of DNA, is capable of initiating the synthesis of a primer extension product in the presence of a suitable polymerising agent. The primer is preferably single-stranded for maximum efficiency in amplification but may alternatively be double-stranded. A primer must be sufficiently long to prime the synthesis of extension products in the presence of the polymerisation agent. The length of the primer depends on many factors, including application, temperature to be employed, template reaction conditions, other reagents, and source of primers. For example, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15 to 35 or more nucleotides, although it may contain fewer nucleotides. Primers can be large polynucleotides, such as from about 200 nucleotides to several kilobases or more. Primers may be selected to be "substantially complementary" to the sequence on the template to which it is designed to hybridise and serve as a site for the initiation of synthesis. By "substantially complementary", it is meant that the primer is sufficiently complementary to hybridise with a target nucleotide sequence. Preferably, the primer contains no mismatches with the template to which it is designed to hybridise but this is not essential. For example, non-complementary nucleotides may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the template. Alternatively, non-complementary nucleotides or a stretch of non-complementary nucleotides can be interspersed into a primer, provided that the primer sequence has sufficient complementarity with the sequence of the template to hybridise therewith and thereby form a template for synthesis of the extension product of the primer.

"*Probe*" refers to a molecule that binds to a specific sequence or sub-sequence or other moiety of another molecule. Unless otherwise indicated, the term "probe" typically refers to a polynucleotide probe that binds to another nucleic acid, often called the "target nucleic acid", through complementary base pairing. Probes may bind target nucleic acids lacking complete sequence complementarity with the probe, depending on the stringency of the hybridisation conditions. Probes can be labelled directly or indirectly.

The term *"recombinant polynucleotide"* as used herein refers to a polynucleotide formed *in vitro* by the manipulation of nucleic acid into a form not normally found in nature. For example, the recombinant polynucleotide may be in the form of an expression vector. Generally, such expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleotide sequence.

By *"recombinant polypeptide"* is meant a polypeptide made using recombinant techniques, *i.e.*, through the expression of a recombinant polynucleotide.

By *"reporter molecule"* as used in the present specification is meant a molecule that, by its chemical nature, provides an analytically identifiable signal that allows the detection of a complex comprising an antigen-binding molecule and its target antigen. The term "reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity" and "substantial identity". A "*reference sequence*" is at least 12 but frequently 15 to 18 and often at least 25 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (*i.e.*, only a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "*comparison window*" refers to a conceptual segment of at least 6 contiguous positions, usually about 50 to about 100, more usually about 100 to about 150 in which a sequence is compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. The comparison window may comprise additions or deletions (*i.e.*, gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerised implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release

7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (*i.e.*, resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul *et al.*, 1997, *Nucl. Acids Res.* 25:3389. A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel *et al.*, "Current Protocols in Molecular Biology", John Wiley & Sons Inc, 1994-1998, Chapter 15.

The term *"sequence identity"* as used herein refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a *"percentage of sequence identity"* is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, I) or the identical amino acid residue (*e.g.*, Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. For the purposes of the present invention, *"sequence identity"* will be understood to mean the "match percentage", calculated by the DNASIS computer program (Version 2.5 for windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, California, USA) using standard defaults as used in the reference manual accompanying the software.

"Stringency" as used herein, refers to the temperature and ionic strength conditions, and presence or absence of certain organic solvents, during hybridisation and washing procedures. The higher the stringency, the higher will be the degree of complementarity between immobilised target nucleotide sequences and the labelled probe polynucleotide sequences that remain hybridised to the target after washing.

"Stringent conditions" refers to temperature and ionic conditions under which only nucleotide sequences having a high frequency of complementary bases will hybridise. The stringency required is nucleotide sequence dependent and depends upon the various components present during hybridisation and subsequent washes, and the time allowed for these processes. Generally, in order to maximise the hybridisation rate, non-stringent

hybridisation conditions are selected; about 20 to 25 °C lower than the thermal melting point (T_m). The T_m is the temperature at which 50% of specific target sequence hybridises to a perfectly complementary probe in solution at a defined ionic strength and pH. Generally, in order to require at least about 85% nucleotide complementarity of hybridised sequences, highly stringent washing conditions are selected to be about 5 to 15 °C lower than the T_m. In order to require at least about 70% nucleotide complementarity of hybridised sequences, moderately stringent washing conditions are selected to be about 15 to 30 °C lower than the T_m. Highly permissive (low stringency) washing conditions may be as low as 50 °C below the T_m, allowing a high level of mis-matching between hybridised sequences. Those skilled in the art will recognise that other physical and chemical parameters in the hybridisation and wash stages can also be altered to affect the outcome of a detectable hybridisation signal from a specific level of homology between target and probe sequences. Other examples of stringency conditions are described in section 3.3.

By *"vector"* is meant a nucleic acid molecule, preferably a DNA molecule derived, for example, from a plasmid, bacteriophage, or plant virus, into which a nucleic acid sequence may be inserted or cloned. A vector preferably contains one or more unique restriction sites and may be capable of autonomous replication in a defined host cell including a target cell or tissue or a progenitor cell or tissue thereof, or be integrable with the genome of the defined host such that the cloned sequence is reproducible. Accordingly, the vector may be an autonomously replicating vector, *i.e.*, a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.*, a linear or closed circular plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into a cell, is integrated into the genome of the recipient cell and replicated together with the chromosome(s) into which it has been integrated. A vector system may comprise a single vector or plasmid, two or more vectors or plasmids, which together contain the total DNA to be introduced into the genome of the host cell, or a transposon. The choice of the vector will typically depend on the compatibility of the vector with the cell into which the vector is to be introduced. The vector may also include a selection marker such as an antibiotic resistance gene that can be used for selection of suitable transformants. Examples of such resistance genes are well known to those of skill in the art.

As used herein, underscoring or italicising the name of a gene shall indicate the gene, in contrast to its protein product, which is indicated by the name of the gene in the absence of any underscoring or italicising. For example, "*xabB*" shall mean the *xabB* gene, whereas "XabB" shall indicate the protein product of the "*xabB*" gene.

2. *Isolated polypeptides, biologically active fragments, polypeptide variants and derivatives*

2.1 Polypeptides of the invention

2.1.1 Albicidin synthetase

The present inventor has also isolated a gene (*xabB*) encoding a large modular polyketide synthase (PKS) linked to a non-ribosomal peptide synthetase (NRPS) (predicted *Mr* 525,695). At 4801 amino acids in length, the product of *xabB* (XabB) is the largest reported PKS-NRPS. Comparison of XabB with available protein sequence databases reveals an N-terminal region (from Met-1 to Asp-3235) similar to many microbial modular PKSs, and a C-terminal region (from Pro-3236 to Asp-4801) similar to NRPSs. Recognisable PKS domains commencing at the N-terminus of XabB, are an acyl-CoA ligase (AL), acyl carrier protein (ACP1), β-ketoacyl synthase (KS1), and β-ketoacyl reductase (KR), followed by two consecutive ACPs and one KS (Figure 1). The motifs characteristic of these domains are aligned with those from other organisms in Figure 3. The AL domain shows 22-30% identity and 50-60% similarity to prokaryotic and eukaryotic aromatic acid-CoA ligases and long-chain fatty acid-CoA ligases, and contains the conserved adenylation core sequence (SGSSG) and the ATPase motif (TGD). The three ACP domains show up to 39.2% identity and 78.6% similarity to acyl carrier proteins, and all contain a 4'-phosphopantetheinyl binding cofactor box GxDS(I/L) (Hopwood and Sherman, 1990), except that A replaces G in ACP1 (Figure 3). The two KS domains show up to 56.1% identity and 80.8% similarity to β-ketoacyl synthases. Both contain motif GPxxxxxxxCSxSL around the active site Cys, and two His residues downstream of the active site Cys, in motifs characteristic of these enzymes (Donadio *et al.*, 1991; Hopwood, 1997; Huang *et al.*, 1998). The KR domain shows up to 27.9% identity and 61.8% similarity to β-ketoacyl reductases, and contains the NAD(P)H binding site GGxGxLG (Scrutton *et al.*, 1990).

At the C-terminus of XabB is an apparent peptide synthetase region linked to the PKS module *via* a peptidyl carrier protein (PCP) domain (Figure 1). The peptide synthetase region shows 31-38% identity and 60-63% similarity with members of the peptide synthetase family. It displays the ordered condensation, adenylation, and PCP domains typical of such multienzymes (Marahiel *et al.*, 1997) followed by an extra condensation domain. The conserved sequences, characteristic of the domains commonly found in peptide synthetases, are compared with those from XabB in Table 2.

In more detail, the full-length amino acid sequence of the *X. albilineans* PKS-NRPS, presented in SEQ ID NO: 2, extends 4801 residues and includes the following sequence signature motifs:

(a) acyl-CoA ligase (AL) motif I extending from about residue 226 to about residue 240, and motif II extending from about residue 486 to about residue 493;

(b) β-ketoacyl synthase 1 (KS1) motif I extending from about residue 897 to about residue 913, motif II extending from about residue 1038 to about residue 1047, and motif III extending from about residue 1080 to about residue 1089;

(c) β-ketoacyl synthase 2 (KS2) motif I extending from about residue 2777 to about residue 2793, motif II extending from about residue 2918 to about residue 2927, and motif III extending from about residue 2955 to about residue 2964;

(d) β-ketoacyl reductase (KR) motif extending from about residue 1812 to about residue 1842;

(e) acyl carrier protein 1 (ACP1) motif extending from about residue 667 to about residue 678;

(f) acyl carrier protein 2 (ACP2) motif extending from about residue 2484 to about residue 2495;

(g) acyl carrier protein 3 (ACP3) motif extending from about residue 2568 to about residue 2579;

(h) adenylation domain (A) motif I extending from about residue 3806 to about residue 3811, motif II extending from about residue 3851 to about residue 3861, motif III extending from about residue 3917 to about residue 3932; motif IV extending from about residue 3967 to about residue 3970, motif V extending from about residue 4063 to about residue 4069, motif VI extending from about residue 4114 to about residue 4128, motif VII extending from about residue 4152 to about residue 4157, motif VIII

extending from about residue 4170 to about residue 4189, motif IX extending from about residue 4239 to about residue 4245, and motif X extending from about residue 4259 to about residue 4264;

(i) peptidyl carrier protein 1 (PCP1) motif extending from about residue 3261 to about residue 3271;

(j) peptidyl carrier protein 2 (PCP2) motif extending from about residue 4306 to about residue 4316;

(k) condensation domain 1 (C1) motif I extending from about residue 3333 to about residue 3342, motif II extending from about residue 3381 to about residue 3389, and motif III extending from about residue 3456 to about residue 3465, motif IV extending from about residue 3495 to about residue 3501, motif V extending from about residue 3606 to about residue 3617, motif VI extending from about residue 3641 to about residue 3647, motif VII extending from about residue 3658 to about residue 3665; and

(l) condensation domain 2 (C2) motif I extending from about residue 4374 to about residue 4383, motif II extending from about residue 4421 to about residue 4429, and motif III extending from about residue 4498 to about residue 4507, motif IV extending from about residue 4538 to about residue 4544, motif V extending from about residue 4649 to about residue 4659, motif VI extending from about residue 4685 to about residue 4691, motif VII extending from about residue 4701 to about residue 4708.

From the above signature motifs, it can be deduced that XabB commences with an AL domain (residues 1-629) followed by an ACP domain (ACP1, residues 630-731). In other PKS systems, an N-terminal AL is involved in activation and incorporation of 3,4-dihydroxycyclohexane carboxylic acid, 3-amino-5-hydroxy benzoic acid (AHBA), or long-chain fatty acid as a starter (Aparicio *et al.*, 1996; Motamedi and Shafiee, 1998; Tang *et al.*, 1998; Duitman *et al.*, 1999). The second module in XabB contains a KS (residues 732-1165), and a KR (residues 1811-1971) upstream of two ACPs (residues 2457-2522, 2544-2613), but lacks any discernable AT domain (Figure 1). The third module contains a KS (residues 2630-3046) followed by a PCP (residues 3221-3307) at the start of the XabB NRPS region.

Four other fused PKS/NRPS systems (Albertini *et al.*, 1995; Gehring *et al.*, 1998; Duitman *et al.*, 1999; Paitan *et al.*, 1999) are known, three of which lack recognisable AT domains (Figure 6). *Yersinia pestis* HMWP1 contains a typical PKS elongation module

(including AT), and an NRPS module with a terminating TE domain. It is the third protein, following an AL (YbtE) and NRPS (HMWP2) in the biosynthetic apparatus for yersiniabactin (Gehring *et al.*, 1998). *B. subtilis* MycA bears the closest resemblance to XabB, showing PKS initiation and elongation modules linked *via* an amino transferase (AMT) domain to the NRPS region. In *B. subtilis* PksK and *M. xanthus* Ta1, the NRPS region precedes the PKS region. Separate AT enzymes encoded elsewhere in the genome may operate in *trans* to load the appropriate acyl groups onto the ACPs in the elongation modules of these PKSs. Candidates are a malonyl-CoA tranascylase gene (*fenF*) located immediately upstream of *mycA* (Duitman *et al.*, 1999), and an acyltransferase gene located 20 kb upstream of *ta1* (Paitan *et al.*, 1999). Accordingly, it is believed that one or more such *trans*-acting AT enzymes may also be involved in connection with the operation of XabB.

From the characteristics of albicidin, and the architecture of the XabB PKS region (Figure 1), the inventor considers that: (i) the AL couples coenzyme A to a shikimate-derived acyl residue in an ATP-dependent reaction, and loads the activated acyl unit onto the 4'-phosphopantetheine prosthetic arm of ACP1; (ii) an acyl group is loaded onto ACP2 or ACP3 by a separate acyltransferase; (iii) the KS1 domain accepts the acyl residue from ACP1 onto a conserved cysteine residue, then transfers it by decarboxylative condensation onto the acyl group tethered to ACP2 or ACP3; (iv) the tethered chain is modified by KR; (v) the assembled polyketide intermediate is translocated *via* KS2 onto the 4-phosphopantetheine prosthetic arm of PCP1, at the start of the NRPS region.

The A domain in the NRPS region of XabB contains ten conserved sequences (A1 to A10, Table 2) identified as AMP, ATP-Mg binding, adenine binding or ATPase sites (Turgay *et al.*, 1992; Marahiel *et al.*, 1997). In other NRPS systems, A domains select and load a particular amino acid, nonproteinogenic amino, hydroxyl or carboxy acid (Marahiel *et al.*, 1997). Substrate specificity is determined at the binding pocket, consisting of a stretch of about 100 amino acid residues between highly conserved motif A4 and A5 (Conti *et al.*, 1997). Sequence alignments for this region reveal some clusters corresponding with the loaded substrate (Stachelhaus *et al.*, 1999). The A domain from XabB falls in a diverse cluster of NRPS modules involved in loading of His, Leu or aromatic amino acids (Phe and Tyr) in other NRPS systems (Figure 7). Based on the architecture of the XabB NPRS region, it can be inferred that the polyketide intermediate

tethered on PCP1 is accepted by C1 and coupled to the amino, hydroxyl, or carboxy acid preloaded by A onto PCP2. The final condensation domain at the C-terminus of XabB is probably involved in peptide-chain termination and cyclisation, as in enniatin, HC-toxin, rapamycin and FK506 systems (Konz and Marahiel, 1999).

2.1.2 Phosphopantetheinyl transferase associated with albicidin biosynthesis

The present invention also provides a gene (*xabA*) from *X. albilineans* encoding a phosphopantetheinyl transferase (PPTase) associated with XabB function. In this regard, XabB contains five carrier protein (ACP/PCP) domains, to which the growing polyketide or polypeptide chain could be covalently tethered. Each functional ACP or PCP domain must have a specific serine side chain phosphopantetheinylated by a dedicated PPTase (Lambalot *et al.*, 1996). The product of *xabA* (XabA) fulfils this function and is required for post-translational activation of synthetases in the albicidin biosynthetic pathway.

The full-length amino acid sequence of this *X. albilineans* PPTase, presented in SEQ ID NO: 83, extends 278 residues and includes the sequence signature motifs for PPTases which are located as follows: (I) motif I spanning from about residue 159 to about residue 167; and (II) motif II spanning from about residue 207 to about residue 218, of SEQ ID NO: 83. The sequence intervening between the two motifs extends from about residue 168 to about residue 206 of SEQ ID NO: 83. These conserved sequence motifs and the intervening sequence are presented for convenience in SEQ ID NO: 89, 93 and 91, respectively.

The deduced *xabA* gene product has 56-62 % overall similarity to EntD proteins for enterobactin biosynthesis and 39-56 % overall similarity to other enzymes in the phosphopantetheinyl transferase superfamily. Like *entD*, *xabA* includes rarely used codons, which may impose post-transcriptional control on the rate of gene product formation (Coderre & Earhart, 1989). Codon optimisation of *xabA* may, therefore, be useful for enhancing the production of XabA.

2.1.3 Methyltransferase associated with albicidin biosynthesis

The invention also provides a gene (*xabC*) from *X. albilineans* encoding a methyltransferase enzyme, more particularly an *O*-methyltransferase enzyme, which is

required for albicidin production and which when expressed above natural levels leads to increased levels and/or functional activities of albicidin antibiotics. The full-length amino acid sequence of this *X. albilineans* methyltransferase, presented in SEQ ID NO: 95, extends 343 residues and includes methyltransferase consensus sequence motifs which are located as follows: (I) motif I spanning from about residue 173 to about residue 180; (II) motif II spanning from about residue 236 to about residue 243; and (III) motif III spanning from about residue 266 to about residue 274, of SEQ ID NO: 95. These conserved sequence motifs are presented for convenience in SEQ ID NO: 99, 101 and 103, respectively.

2.2 Biologically active fragments

The invention also contemplates biological fragments of the above polypeptides of at least 6 and preferably at least 8 amino acids in length, which comprise an activity associated with the domains described above. For example, biologically active fragments may be produced according to any suitable procedure known in the art. For example, a suitable method may include first producing a fragment of a parent polypeptide as described in Section 2.1 and then testing the fragment for the appropriate biological activity. In one embodiment, the fragment is derived from the albicidin PKS-NRPS of the invention and is tested for an activity selected form the group consisting of acyl-CoA ligase activity, β-ketoacyl synthase activity, β-ketoacyl reductase, acyl carrier protein activity, adenylation activity, peptidyl carrier protein activity and condensation activity.

Any assays that detects or preferably measure such activities is contemplated in the practice of the present invention. The biologically active fragment suitably comprises any one or more of the sequence signature motifs described above, or variants thereof. Preferably, the biologically active fragment comprises all said sequence signature motifs, or variants thereof.

In another embodiment, the fragment is derived from the PPTase of the invention and is tested for PPTase activity according to standard assays known to persons of skill in the art. Suitably, the PPTase catalyses the pantetheinylation, more preferably the phosphopantetheinylation, of proteins involved in antibiotic biosynthesis, preferably albicidin biosynthesis. The biologically active fragment preferably comprises the consensus sequence motifs set forth in SEQ ID NO: 89 and 93, or variant thereof and thus,

more preferably comprises the sequence from about residue 159 to about residue 218, of SEQ ID NO: 83.

In yet another embodiment, the fragment is derived from the methyltransferase of the invention and is tested for methyltransferase activity, preferably *O*-methyltransferase activity and more preferably *S*-adenosylmethionine-dependent *O*-methyltransferase activity. Suitably, the methyltransferase catalyses the transfer of one or more methyl groups to an antibiotic precursor, more preferably an albicidin precursor or an intermediate relating to the biosynthesis of albicidins. The biologically active fragment preferably comprises the consensus sequence motifs set forth in SEQ ID NO: 99, 101 and 103, or variant thereof and thus, more preferably comprises the sequence from about residue 173 to about residue 274 of SEQ ID NO: 95 (*i.e.*, SEQ ID NO: 105), or variant of said sequence. In an especially preferred embodiment, the biologically active fragment comprises the sequence from about residue 1 to about residue 277 of SEQ ID NO: 95 (*i.e.*, SEQ ID NO: 107), or variant of said sequence. An exemplary polynucleotide encoding this sequence is cloned in vector pLXABB described *infra*.

Alternatively, biological activity of the fragment is tested by introducing a polynucleotide from which a fragment of a parent polypeptide can be translated into a cell, and detecting one or more of the above activities, which is indicative of said fragment being a biologically active fragment. In one embodiment, such activity can be assayed by introducing into an albicidin deficient *xabB*⁻ *X. albilineans* mutant (*e.g.*, strain LS157 described herein) a polynucleotide from which a PKS-NRPS-associated fragment can be produced and assaying for antibiotic activity using a microbial plate assay, as for instance described in Example 1.

In another embodiment embodiment, PPTase activity is assayed by introducing into an albicidin deficient *xabA*⁻ *X. albilineans* mutant (*e.g.*, strain LS156 described herein) a polynucleotide from which a PPTase-associated fragment can be produced and assaying for antibiotic activity using a microbial plate assay, as for instance described in Example 2.

In yet another embodiment, methyltransferase activity is assayed by introducing into an albicidin deficient *xabC*⁻ *X. albilineans* mutant (*e.g.*, strain LS-JP1 described herein) a polynucleotide from which a methyltransferase-associated fragment can be

produced and assaying for antibiotic activity as for example described herein using a microbial plate assay, as for instance described in Example 3.

2.3 Polypeptide variants

The invention also contemplates polypeptide variants of the polypeptides of the invention wherein said variants have an activity selected form the group consisting of acyl-CoA ligase activity, β-ketoacyl synthase activity, β-ketoacyl reductase, acyl carrier protein activity, adenylation activity, peptidyl carrier protein activity, condensation activity, PPTase activity, and methyltransferase activity. Suitable methods of producing polypeptide variants include, for example, producing a modified polypeptide whose sequence is distinguished from a parent polypeptide as described in Section 2.1 or a biologically active fragment thereof by the substitution, deletion and/or addition of at least one amino acid. The modified polypeptide is then tested for one or more of said activities, wherein the presence of that activity indicates that the modified polypeptide is a variant of the parent polypeptide.

In another embodiment, a polypeptide variant is produced by introducing into a cell a polynucleotide from which a modified polypeptide can be translated, and detecting one or more of the activities described above that are associated with the cell, which is indicative of the modified polypeptide being a polypeptide variant.

In general, variants will have at least 60%, more suitably at least 70%, preferably at least 80%, and more preferably at least 90% homology to a polypeptide as for example shown in SEQ ID NO: 4, or a biological fragment thereof. It is preferred that variants display at least 60%, more suitably at least 70%, preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90% and still more preferably at least 95% sequence identity with a parent polypeptide as described in Section 2.1 or a biologically active fragment thereof. In this respect, the window of comparison preferably spans about the full length of the polypeptide or of the biologically active fragment. Suitable variants can be obtained from any secondary metabolite-producing organism, and preferably from an albicidin-producing organism.

Alternatively polypeptide variants according to the invention can be identified either rationally, or *via* established methods of mutagenesis (see, for example, Watson, J.

D. *et al.*, "MOLECULAR BIOLOGY OF THE GENE", Fourth Edition, Benjamin/Cummings, Menlo Park, Calif., 1987). Significantly, a random mutagenesis approach requires no *a priori* information about the gene sequence that is to be mutated. This approach has the advantage that it assesses the desirability of a particular mutant based on its function, and thus does not require an understanding of how or why the resultant mutant protein has adopted a particular conformation. Indeed, the random mutation of target gene sequences has been one approach used to obtain mutant proteins having desired characteristics (Leatherbarrow, R. 1986, *J. Prot. Eng.* **1**: 7-16; Knowles, J. R., 1987, *Science* **236**: 1252-1258; Shaw, W. V., 1987, *Biochem. J.* **246**: 1-17; Gerit, J. A. 1987, *Chem. Rev.* **87**: 1079-1105). Alternatively, where a particular sequence alteration is desired, methods of site-directed mutagenesis can be employed. Thus, such methods may be used to selectively alter only those amino acids of the protein that are believed to be important (Craik, C. S., 1985, *Science* **228**: 291-297; Cronin, *et al.*, 1988, *Biochem.* **27**: 4572-4579; Wilks, *et al.*, 1988, *Science* **242**: 1541-1544).

Variant peptides or polypeptides, resulting from rational or established methods of mutagenesis or from combinatorial chemistries may comprise conservative amino acid substitutions. Exemplary conservative substitutions in a polypeptide or polypeptide fragment according to the invention may be made according to the following table.

TABLE B

Original Residue	*Exemplary Substitutions*
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro

Original Residue	*Exemplary Substitutions*
His	Asn, Gln
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile,
Phe	Met, Leu, Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp, Phe
Val	Ile, Leu

Substantial changes in function are made by selecting substitutions that are less conservative than those shown in TABLE 3. Other replacements would be non-conservative substitutions and relatively fewer of these may be tolerated. Generally, the substitutions which are likely to produce the greatest changes in a polypeptide's properties are those in which (a) a hydrophilic residue (*e.g.*, Ser or Asn) is substituted for, or by, a hydrophobic residue (*e.g.*, Ala, Leu, Ile, Phe or Val); (b) a cysteine or proline is substituted for, or by, any other residue; (c) a residue having an electropositive side chain (*e.g.*, Arg, His or Lys) is substituted for, or by, an electronegative residue (*e.g.*, Glu or Asp) or (d) a residue having a smaller side chain (*e.g.*, Ala, Ser) or no side chain (*e.g.*, Gly) is substituted for, or by, one having a bulky side chain (*e.g.*, Phe or Trp).

2.4 Polypeptide derivatives

A polypeptide can typically tolerate one or more amino acid deletions and insertions in its amino acid sequence without loss or significant loss of a desired activity. Accordingly, the invention also contemplates derivatives of the parent polypeptides of the invention described in Section 2.1 or biologically active fragments thereof or variants of

these, which include amino acid deletions and/or additions, wherein said derivatives comprise one or more activities selected form the group consisting of acyl-CoA ligase activity, β-ketoacyl synthase activity, β-ketoacyl reductase, acyl carrier protein activity, adenylation activity, peptidyl carrier protein activity, condensation activity, PPTase activity and methyltransferase activity associated with antibiotic biosynthesis, and preferably with albicidin biosynthesis.

Preferred derivatives of the invention include PKS-NRPS molecules with altered activities in one or more respects and thus produce polyketides other than the albicidin natural product(s) of the XabB. A PKS-NRPS derived from XabB by such alteration includes a modular PKS-NRPS (or its corresponding encoding gene(s)) that retains the scaffolding of the utilised portion encoded by the naturally occurring gene. Not all domains or modules need be altered. On the constant scaffold, at least one enzymatic activity is mutated, deleted, replaced, or inserted so as to alter the activity of the resulting PKS-NRPS relative to the original or parent PKS-NRPS. Alteration results when these activities are deleted or are replaced by a different version of the activity, or simply mutated in such a way that a polyketide other than the natural product results from these collective activities. This occurs because there has been a resulting alteration of the starter unit and/or elongation unit, stereochemistry, chain length or cyclisation, and/or reductive dehydration cycle outcome at a corresponding position in the product polyketide. Where a deleted activity is replaced, the origin of the replacement activity may come from a corresponding activity in a different naturally occurring PKS or PKS-NRPS or from a different region of the albicidin PKS-NRPS. Any or all PKS/NRPS genes may be included in the derivative or portions of any of these may be included, but the scaffolding of the albicidin PKS-NRPS protein is preferably retained in whatever derivative is constructed.

Thus, a PKS-NRPS derived from the albicidin PKS-NRPS includes a PKS-NRPS that contains the scaffolding of all or a portion of XabB. The derived PKS-NRPS also contains at least two elongation modules that are functional and preferably at least three elongation modules. The derived PKS-NRPS also contains mutations, deletions, insertions, or replacements of one or more of the activities of the functional domains or modules of XabB so that the nature of the resulting polyketide is altered. Exemplary embodiments include those wherein a KS or ACP domain has been deleted or replaced by a version of the activity from a different PKS/NRPS or from another location within XabB. Also

contemplated are derivatives where at least one non-condensation cycle enzymatic activity (KR, KR, or A) has been deleted or added or wherein any of these activities has been mutated so as to change the structure of the polyketide synthesised by the PKS.

Other derivatives contemplated by the present invention include fusion of the polypeptides, fragments and polypeptide variants of the invention with other polypeptides or proteins. For example, it will be appreciated that said polypeptides, fragments or variants may be incorporated into larger polypeptides, and that such larger polypeptides may also be expected to have one or more of the activities mentioned above. The polypeptides, fragments or variants of the invention may be fused to a further protein, for example, which is not derived from the original host. The further protein may assist in the purification of the fusion protein. For instance, a polyhistidine tag or a maltose binding protein may be used in this respect as described in more detail below. Other possible fusion proteins are those which produce an immunomodulatory response. Particular examples of such proteins include Protein A or glutathione S-transferase (GST).

Other derivatives contemplated by the invention include, but are not limited to, modification to side chains, incorporation of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the polypeptides, fragments and variants of the invention. Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by acylation with acetic anhydride; acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; amidination with methylacetimidate; carbamoylation of amino groups with cyanate; pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with $NaBH_4$; reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; and trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS). The carboxyl group may be modified by carbodiimide activation via O-acylisourea formation followed by subsequent derivatisation, by way of example, to a corresponding amide. The guanidine group of arginine residues may be modified by formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal. Sulphydryl groups may be modified by methods such as performic acid oxidation to cysteic acid; formation of mercurial derivatives using 4-chloromercuriphenylsulphonic acid, 4-chloromercuribenzoate; 2-chloromercuri-4-

nitrophenol, phenylmercury chloride, and other mercurials; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; carboxymethylation with iodoacetic acid or iodoacetamide; and carbamoylation with cyanate at alkaline pH. Tryptophan residues may be modified, for example, by alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphonyl halides or by oxidation with N-bromosuccinimide. Tyrosine residues may be modified by nitration with tetranitromethane to form a 3-nitrotyrosine derivative. The imidazole ring of a histidine residue may be modified by N-carbethoxylation with diethylpyrocarbonate or by alkylation with iodoacetic acid derivatives.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include but are not limited to, use of 4-amino butyric acid, 6-aminohexanoic acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 4-amino-3-hydroxy-6-methylheptanoic acid, t-butylglycine, norleucine, norvaline, phenylglycine, ornithine, sarcosine, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acids contemplated by the present invention is shown in TABLE C.

TABLE C

Non-conventional amino acid	*Non-conventional amino acid*
α-aminobutyric acid	L-N-methylalanine
α-amino-α-methylbutyrate	L-N-methylarginine
aminocyclopropane-carboxylate	L-N-methylasparagine
aminoisobutyric acid	L-N-methylaspartic acid
aminonorbornyl-carboxylate	L-N-methylcysteine
cyclohexylalanine	L-N-methylglutamine
cyclopentylalanine	L-N-methylglutamic acid
L-N-methylisoleucine	L-N-methylhistidine
D-alanine	L-N-methylleucine
D-arginine	L-N-methyllysine
D-aspartic acid	L-N-methylmethionine

Non-conventional amino acid	*Non-conventional amino acid*
D-cysteine	L-N-methylnorleucine
D-glutamate	L-N-methylnorvaline
D-glutamic acid	L-N-methylornithine
D-histidine	L-N-methylphenylalanine
D-isoleucine	L-N-methylproline
D-leucine	L-N-medlylserine
D-lysine	L-N-methylthreonine
D-methionine	L-N-methyltryptophan
D-ornithine	L-N-methyltyrosine
D-phenylalanine	L-N-methylvaline
D-proline	L-N-methylethylglycine
D-serine	L-N-methyl-t-butylglycine
D-threonine	L-norleucine
D-tryptophan	L-norvaline
D-tyrosine	α-methyl-aminoisobutyrate
D-valine	α-methyl-γ-aminobutyrate
D-α-methylalanine	α-methylcyclohexylalanine
D-α-methylarginine	α-methylcylcopentylalanine
D-α-methylasparagine	α-methyl-α-napthylalanine
D-α-methylaspartate	α-methylpenicillamine
D-α-methylcysteine	N-(4-aminobutyl)glycine
D-α-methylglutamine	N-(2-aminoethyl)glycine
D-α-methylhistidine	N-(3-aminopropyl)glycine
D-α-methylisoleucine	N-amino-α-methylbutyrate
D-α-methylleucine	α-napthylalanine

Non-conventional amino acid	*Non-conventional amino acid*
D-α-methyllysine	N-benzylglycine
D-α-methylmethionine	N-(2-carbamylediyl)glycine
D-α-methylornithiine	N-(carbamylmethyl)glycine
D-α-methylphenylalanine	N-(2-carboxyethyl)glycine
D-α-methylproline	N-(carboxymethyl)glycine
D-α-methylserine	N-cyclobutylglycine
D-α-methylthreonine	N-cycloheptylglycine
D-α-methyltryptophan	N-cyclohexylglycine
D-α-methyltyrosine	N-cyclodecylglycine
L-α-methylleucine	L-α-methyllysine
L-α-methylmethionine	L-α-methylnorleucine
L-α-methylnorvatine	L-α-methylornithine
L-α-methylphenylalanine	L-α-methylproline
L-α-methylserine	L-α-methylthreonine
L-α-methyltryptophan	L-α-methyltyrosine
L-α-methylvaline	L-N-methylhomophenylalanine
N-(N-(2,2-diphenylethyl carbamylmethyl)glycine	N-(N-(3,3-diphenylpropyl carbamylmethyl)glycine
1-carboxy-1-(2,2-diphenyl-ethyl amino)cyclopropane	

Also contemplated is the use of crosslinkers, for example, to stabilise 3D conformations of the polypeptides, fragments or variants of the invention, using homo-bifunctional cross linkers such as bifunctional imido esters having $(CH_2)_n$ spacer groups with n = 1 to n = 6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety or

carbodiimide. In addition, peptides can be conformationally constrained, for example, by introduction of double bonds between C_α and C_β atoms of amino acids, by incorporation of C_α and N_α-methylamino acids, and by formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini between two side chains or between a side chain and the N or C terminus of the peptides or analogues. For example, reference may be made to: Marlowe (1993, *Biorganic & Medicinal Chemistry Letters* 3: 437-44) who describes peptide cyclisation on TFA resin using trimethylsilyl (TMSE) ester as an orthogonal protecting group; Pallin and Tam (1995, *J. Chem. Soc. Chem. Comm.* 2021-2022) who describe the cyclisation of unprotected peptides in aqueous solution by oxime formation; Algin *et al* (1994, *Tetrahedron Letters* 35: 9633-9636) who disclose solid-phase synthesis of head-to-tail cyclic peptides *via* lysine side-chain anchoring; Kates *et al* (1993, *Tetrahedron Letters* 34: 1549-1552) who describe the production of head-to-tail cyclic peptides by three-dimensional solid phase strategy; Tumelty *et al* (1994, *J. Chem. Soc. Chem. Comm.* 1067-1068) who describe the synthesis of cyclic peptides from an immobilised activated intermediate, wherein activation of the immobilised peptide is carried out with *N*-protecting group intact and subsequent removal leading to cyclisation; McMurray *et al* (1994, *Peptide Research* 7: 195-206) who disclose head-to-tail cyclisation of peptides attached to insoluble supports by means of the side chains of aspartic and glutamic acid; Hruby *et al* (1994, *Reactive Polymers* 22: 231-241) who teach an alternate method for cyclising peptides *via* solid supports; and Schmidt and Langer (1997, *J. Peptide Res.* 49: 67-73) who disclose a method for synthesising cyclotetrapeptides and cyclopentapeptides. The foregoing methods may be used to produce conformationally constrained polypeptides that comprise one or more activities selected form the group consisting of acyl-CoA ligase activity, β-ketoacyl synthase activity, β-ketoacyl reductase, acyl carrier protein activity, adenylation activity, peptidyl carrier protein activity, condensation activity, PPTase activity and methyltransferase activity associated with the production of polyketides and particularly albicidins or analogues thereof.

The invention also contemplates polypeptides, fragments or variants of the invention that have been modified using ordinary molecular biological techniques so as to improve their resistance to proteolytic degradation or to optimise solubility properties or to render them more suitable as an immunogenic agent.

3. *Polynucleotides of the invention*

3.1 Polynucleotides encoding polypeptides of the invention

3.1.1 Albicidin synthetase-encoding polynucleotides

The invention further provides a polynucleotide that encodes a PKS-NRPS polypeptide of the invention, or biologically active fragment thereof, or variant or derivative of these as defined above. In one embodiment, the polynucleotide comprises the entire sequence of nucleotides set forth in SEQ ID NO: 1. SEQ ID NO: 1 corresponds to a 16511-bp *X. albilineans xabB* cistron. SEQ ID NO: 3, defines the full-length coding sequence of *xabB* and encodes various sequence signature motifs at the following nucleotide positions:

(a) acyl-CoA ligase (AL) motif I from about nucleotide 676 to about nucleotide 720, and motif II from about nucleotide 1456 to about nucleotide 1477;

(b) β-ketoacyl synthase 1 (KS1) motif I from about nucleotide 2689 to about nucleotide 2739, motif II from about nucleotide 3112 to about nucleotide 3141, and motif III from about nucleotide 3238 to about nucleotide 3267;

(c) β-ketoacyl synthase 2 (KS2) motif I from about nucleotide 8329 to about nucleotide 8379, motif II from about nucleotide 8752 to about nucleotide 8781, and motif III from about nucleotide 8863 to about nucleotide 8892;

(d) β-ketoacyl reductase (KR) motif from about nucleotide 5434 to about nucleotide 5526;

(e) acyl carrier protein 1 (ACP1) motif from about nucleotide 1999 to about nucleotide 2034;

(f) acyl carrier protein 2 (ACP2) motif from about nucleotide 7450 to about nucleotide 7485;

(g) acyl carrier protein 3 (ACP3) motif from about nucleotide 7702 to about nucleotide 7735;

(h) adenylation domain (A) motif I from about nucleotide 11416 to about nucleotide 11433, motif II from about nucleotide 11551 to about nucleotide 11583, motif III from about nucleotide 11749 to about nucleotide 11796; motif IV from about nucleotide 11899 to about nucleotide 11910, motif V from about nucleotide 12187 to about nucleotide 12207, motif VI from about nucleotide 12340 to about nucleotide 12384,

motif VII from about nucleotide 12454 to about nucleotide 12471, motif VIII from about nucleotide 12508 to about nucleotide 12567, motif IX from about nucleotide 12715 to about nucleotide 12735, and motif X from about nucleotide 12775 to about nucleotide 12792;

(i) peptidyl carrier protein 1 (PCP1) motif from about nucleotide 9781 to about nucleotide 9813;

(j) peptidyl carrier protein 2 (PCP2) motif from about nucleotide 12916 to about nucleotide 12948;

(k) condensation domain 1 (C1) motif I from about nucleotide 9997 to about nucleotide 10026, motif II from about nucleotide 10141 to about nucleotide 10167, and motif III from about nucleotide 10366 to about nucleotide 10395, motif IV from about nucleotide 10483 to about nucleotide 10503, motif V from about nucleotide 10816 to about nucleotide 10851, motif VI from about nucleotide 10921 to about nucleotide 10941, motif VII from about nucleotide 10972 to about nucleotide 10995; and

(l) condensation domain 2 (C2) motif I from about nucleotide 13120 to about nucleotide 13149, motif II from about nucleotide 13261 to about nucleotide 13287, and motif III from about nucleotide 13492 to about nucleotide 13521, motif IV from about nucleotide 13612 to about nucleotide 13632, motif V from about nucleotide 13945 to about nucleotide 13977, motif VI from about nucleotide 14053 to about nucleotide 14073, motif VII from about nucleotide 14101 to about nucleotide 14124.

Those of skill in the art will recognise that, due to the degenerate nature of the genetic code, a variety of polynucleotides differing in their nucleotide sequences can be used to encode a given amino acid sequence of the invention. The native polynucleotide sequence encoding the PKS-NRPS of *X. albilineans* is shown herein merely to illustrate a preferred embodiment of the invention, and the invention includes polynucleotides of any sequence that encode the amino acid sequences of the polypeptides and proteins of the invention.

3.1.2 PPTase-encoding polynucleotides

The invention further provides a polynucleotide that encodes a PPTase polypeptide of the invention, or biologically active fragment thereof, or variant or derivative of these as defined above. In one embodiment, the polynucleotide comprises the

entire sequence of nucleotides set forth in SEQ ID NO: 82. SEQ ID NO: 82 corresponds to a 1200-bp *X. albilineans xabA* cistron. This sequence encodes a PPTase catalytic domain from about nucleotide 475 to about nucleotide 654. This domain comprises two conserved PPTase sequence motifs: (I) motif I encoded by a nucleotide sequence from about nucleotide 475 to about nucleotide 501; and (II) motif II encoded by a nucleotide sequence from about nucleotide 619 to about nucleotide 654, of SEQ ID NO: 82. The intervening amino acid sequence, linking motifs 1 and II, is encoded by a nucleotide sequence from about nucleotide 502 to about nucleotide 618 of SEQ ID NO: 82. The said nucleotide sequences are presented for convenience in SEQ ID NO: 86, 88, 92 and 90, respectively. Suitably, the polynucleotide comprises the sequence set forth in SEQ ID NO: 84, which defines the full-length coding sequence of *xabA*. Alternatively, the polynucleotide comprises a contiguous sequence of nucleotides contained within the sequence set forth in SEQ ID NO: 86, which encodes the PPTase catalytic domain.

3.1.3 Methyltransferase-encoding polynucleotides

The invention further provides a polynucleotide that encodes a methyltransferase polypeptide of the invention, or biologically active fragment thereof, or variant or derivative of these as defined above. In one embodiment, the polynucleotide comprises the entire sequence of nucleotides set forth in SEQ ID NO: 94. SEQ ID NO: 94 corresponds to a 1515-bp *X. albilineans xabC* cistron. This sequence encodes three conserved methyltransferase sequence motifs: (I) motif I encoded by a nucleotide sequence from about nucleotide 565 to about nucleotide 585; (II) motif II encoded by a nucleotide sequence from about nucleotide 741 to about nucleotide 774; and (III) motif III encoded by a nucleotide sequence from about nucleotide 841 to about nucleotide 867, or SEQ ID NO: 94. The said nucleotide sequences are presented for convenience in SEQ ID NO: 98, 100 and 102, respectively. Suitably, the polynucleotide comprises the sequence set forth in SEQ ID NO: 96, which defines the full-length coding sequence of *xabC*. Alternatively, the polynucleotide comprises a contiguous sequence of nucleotides contained within the sequence set forth in SEQ ID NO: 104 or 106, which encode biologically active fragments as described in Section 2.2.

3.2 Polynucleotide variants

In general, polynucleotide variants according to the invention comprise regions that show at least 60%, more suitably at least 70%, preferably at least 80%, and more preferably at least 90% sequence identity over a reference polynucleotide sequence of identical size ("*comparison window*") or when compared to an aligned sequence in which the alignment is performed by a computer homology program known in the art. What constitutes suitable variants may be determined by conventional techniques. For example, a polynucleotide comprising at least one sequence selected from the group consisting of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102 and 104 can be altered using any suitable method including conventional recombinant techniques and mutagenesis methods such as random mutagenesis (*e.g.*, transposon mutagenesis), oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis and cassette mutagenesis of an earlier prepared variant or non-variant version of an isolated polynucleotide of the invention.

Alternatively, polynucleotide sequences variants encoding heterologous PKS/NRPS enzymes for producing PKS-NRPS variants of the invention may be obtained from other secondary metabolite- or polyketide-producing organisms. For example, such variants may be prepared according to the following procedure:

(a) creating primers which are optionally degenerate wherein each comprises a portion of a reference polynucleotide encoding a reference polypeptide or fragment of the invention, preferably encoding at least one sequence selected from the group consisting of SEQ ID NO: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 83, 87, 89, 91, 93, 95, 99, 101, 103, 105 and 107;

(b) obtaining a nucleic acid extract from a secondary metabolite-producing organism, which is preferably a bacterium, more preferably from a species of the family *Pseudomonadaceae*, more preferably from a *Xanthomonas* species; and

(c) using said primers to amplify, *via* nucleic acid amplification techniques, at least one amplification product from said nucleic acid extract, wherein said amplification product corresponds to a polynucleotide variant.

Suitable nucleic acid amplification techniques are well known to the skilled addressee, and include polymerase chain reaction (PCR) as for example described in Ausubel *et al.* (*supra*); strand displacement amplification (SDA) as for example described in U.S. Patent No 5,422,252; rolling circle replication (RCR) as for example described in Liu *et al.*, (1996, *J. Am. Chem. Soc.* **118**:1587-1594 and International application WO 92/01813) and Lizardi *et al.*, (International Application WO 97/19193); nucleic acid sequence-based amplification (NASBA) as for example described by Sooknanan *et al.*, (1994, *Biotechniques* 17:1077-1080); and Q-β replicase amplification as for example described by Tyagi *et al.*, (1996, *Proc. Natl. Acad. Sci. USA* **93**: 5395-5400).

Typically, polynucleotide variants that are substantially complementary to a reference polynucleotide are identified by blotting techniques that include a step whereby nucleic acids are immobilised on a matrix (preferably a synthetic membrane such as nitrocellulose), followed by a hybridisation step, and a detection step. Southern blotting is used to identify a complementary DNA sequence; northern blotting is used to identify a complementary RNA sequence. Dot blotting and slot blotting can be used to identify complementary DNA/DNA, DNA/RNA or RNA/RNA polynucleotide sequences. Such techniques are well known by those skilled in the art, and have been described in Ausubel *et al.* (1994-1998, *supra*) at pages 2.9.1 through 2.9.20.

According to such methods, Southern blotting involves separating DNA molecules according to size by gel electrophoresis, transferring the size-separated DNA to a synthetic membrane, and hybridising the membrane-bound DNA to a complementary nucleotide sequence labelled radioactively, enzymatically or fluorochromatically. In dot blotting and slot blotting, DNA samples are directly applied to a synthetic membrane prior to hybridisation as above. An alternative blotting step is used when identifying complementary polynucleotides in a cDNA or genomic DNA library, such as through the process of plaque or colony hybridisation. A typical example of this procedure is described in Sambrook *et al.* ("Molecular Cloning. A Laboratory Manual", Cold Spring Harbour Press, 1989) Chapters 8-12.

Typically, the following general procedure can be used to determine hybridisation conditions. Polynucleotides are blotted/transferred to a synthetic membrane, as described above. A reference polynucleotide such as a polynucleotide of the invention is labelled as

described above, and the ability of this labelled polynucleotide to hybridise with an immobilised polynucleotide is analysed. A skilled addressee will recognise that a number of factors influence hybridisation. The specific activity of radioactively labelled polynucleotide sequence should typically be greater than or equal to about 10^8 dpm/mg to provide a detectable signal. A radiolabelled nucleotide sequence of specific activity 10^8 to 10^9 dpm/mg can detect approximately 0.5 pg of DNA. It is well known in the art that sufficient DNA must be immobilised on the membrane to permit detection. It is desirable to have excess immobilised DNA, usually 10 μg. Adding an inert polymer such as 10% (w/v) dextran sulfate (MW 500,000) or polyethylene glycol 6000 during hybridisation can also increase the sensitivity of hybridisation (see Ausubel *supra* at 2.10.10).

To achieve meaningful results from hybridisation between a polynucleotide immobilised on a membrane and a labelled polynucleotide, a sufficient amount of the labelled polynucleotide must be hybridised to the immobilised polynucleotide following washing. Washing ensures that the labelled polynucleotide is hybridised only to the immobilised polynucleotide with a desired degree of complementarity to the labelled polynucleotide. It will be understood that polynucleotide variants according to the invention will hybridise to a reference polynucleotide under at least low stringency conditions. Reference herein to low stringency conditions include and encompass from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1 M to at least about 2 M salt for hybridisation at 42° C, and at least about 1 M to at least about 2 M salt for washing at 42° C. Low stringency conditions also may include 1% Bovine Serum Albumin (BSA), 1 mM EDTA, 0.5 M $NaHPO_4$ (pH 7.2), 7% SDS for hybridisation at 65° C, and (i) 2xSSC, 0.1% SDS; or (ii) 0.5% BSA, 1 mM EDTA, 40 mM $NaHPO_4$ (pH 7.2), 5% SDS for washing at room temperature.

Suitably, the polynucleotide variants hybridise to a reference polynucleotide under at least medium stringency conditions. Medium stringency conditions include and encompass from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5 M to at least about 0.9 M salt for hybridisation at 42° C, and at least about 0.1 M to at least about 0.2 M salt for washing at 55° C. Medium stringency conditions also may include 1% Bovine Serum Albumin (BSA), 1 mM EDTA, 0.5 M $NaHPO_4$ (pH 7.2), 7% SDS for hybridisation at 65° C, and (i) 2 x SSC, 0.1% SDS; or (ii) 0.5% BSA, 1 mM EDTA, 40 mM $NaHPO_4$ (pH 7.2), 5% SDS for washing at 60-65° C.

Preferably, the polynucleotide variants hybridise to a reference polynucleotide under high stringency conditions. High stringency conditions include and encompass from at least about 31% v/v to at least about 50% v/v formamide and from about 0.01 M to about 0.15 M salt for hybridisation at 42° C, and about 0.01 M to about 0.02 M salt for washing at 55° C. High stringency conditions also may include 1% BSA, 1 mM EDTA, 0.5 M $NaHPO_4$ (pH 7.2), 7% SDS for hybridisation at 65° C, and (i) 0.2 x SSC, 0.1% SDS; or (ii) 0.5% BSA, 1mM EDTA, 40 mM $NaHPO_4$ (pH 7.2), 1% SDS for washing at a temperature in excess of 65° C.

Other stringent conditions are well known in the art. A skilled addressee will recognise that various factors can be manipulated to optimise the specificity of the hybridisation. Optimisation of the stringency of the final washes can serve to ensure a high degree of hybridisation. For detailed examples, see Ausubel *et al.*, *supra* at pages 2.10.1 to 2.10.16 and Sambrook *et al.* (1989, *supra*) at sections 1.101 to 1.104.

While stringent washes are typically carried out at temperatures from about 42° C to 68° C, one skilled in the art will appreciate that other temperatures may be suitable for stringent conditions. Maximum hybridisation rate typically occurs at about 20° C to 25° C below the T_m for formation of a DNA-DNA hybrid. It is well known in the art that the T_m is the melting temperature, or temperature at which two complementary polynucleotide sequences dissociate. Methods for estimating T_m are well known in the art (see Ausubel *et al.*, *supra* at page 2.10.8).

In general, the T_m of a perfectly matched duplex of DNA may be predicted as an approximation by the formula:

$$T_m = 81.5 + 16.6 (\log_{10} M) + 0.41 (\%G+C) - 0.63 (\% \text{ formamide}) - (600/\text{length})$$

wherein: M is the concentration of Na^+, preferably in the range of 0.01 molar to 0.4 molar; %G+C is the sum of guanosine and cytosine bases as a percentage of the total number of bases, within the range between 30% and 75% G+C; % formamide is the percent formamide concentration by volume; length is the number of base pairs in the DNA duplex.

The T_m of a duplex DNA decreases by approximately 1° C with every increase of 1% in the number of randomly mismatched base pairs. Washing is generally carried out at T_m – 15° C for high stringency, or T_m – 30° C for moderate stringency.

In a preferred hybridisation procedure, a membrane (*e.g.*, a nitrocellulose membrane or a nylon membrane) containing immobilised DNA is hybridised overnight at 42° C in a hybridisation buffer (50% deionised formamide, 5xSSC, 5x Denhardt's solution (0.1% ficoll, 0.1% polyvinylpyrollidone and 0.1% bovine serum albumin), 0.1% SDS and 200 mg/mL denatured salmon sperm DNA) containing labelled probe. The membrane is then subjected to two sequential medium stringency washes (*i.e.*, 2xSSC, 0.1% SDS for 15 min at 45° C, followed by 2xSSC, 0.1% SDS for 15 min at 50° C), followed by two sequential higher stringency washes (*i.e.*, 0.2xSSC, 0.1% SDS for 12 min at 55° C followed by 0.2xSSC and 0.1%SDS solution for 12 min at 65-68° C.

Methods for detecting a labelled polynucleotide hybridised to an immobilised polynucleotide are well known to practitioners in the art. Such methods include autoradiography, phosphorimaging, and chemiluminescent, fluorescent and colorimetric detection.

4. Expression vectors

The present invention further provides expression vectors designed for genetic transformation of cells, preferably prokaryotic cells, comprising a polynucleotide, fragment or variant according to the invention operably linked to a regulatory polynucleotide. An expression vector is typically a nucleic acid that can be introduced into a host cell or cell-free transcription and translation system. An expression vector can be maintained permanently or transiently in a cell, whether as part of the chromosomal or other DNA in the cell or in any cellular compartment, such as a replicating vector in the cytoplasm.

The various components of an expression vector can vary widely, depending on the intended use of the vector and especially the host cell(s) in which the vector is intended to replicate or drive expression. For example, the regulatory polynucleotide, which is used to control expression of a polynucleotide of the invention, will generally be appropriate for the host cell used for expression. Numerous types of appropriate expression vectors and suitable regulatory sequences are known in the art for a variety of host cells. Typically, the

regulatory polynucleotide includes, but is not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and termination sequences, and enhancer or activator sequences. Constitutive or inducible promoters as known in the art are contemplated by the invention. The promoters may be either naturally occurring promoters, or hybrid promoters that combine elements of more than one promoter.

In a preferred embodiment, the expression vector is operable in a Gram-negative prokaryotic cell. A variety of prokaryotic expression vectors, which may be used as a basis for constructing the expression vector of the invention. These include but are not limited to a chromosomal vector (*e.g.*, a bacteriophage such as bacteriophage λ), an extrachromosomal vector (*e.g.*, a plasmid or a cosmid expression vector). The expression vector will also typically contain an origin of replication, which allows autonomous replication of the vector, and one or more selectable marker genes that allow phenotypic selection of the transformed cells.

The expression vector may also include a fusion partner (typically provided by the expression vector) so that a recombinant polypeptide is expressed as a fusion polypeptide with said fusion partner. The main advantage of fusion partners is that they assist identification and/or purification of said fusion polypeptide. In order to express said fusion polypeptide, it is necessary to ligate a polynucleotide according to the invention into the expression vector so that the translational reading frames of the fusion partner and the polynucleotide coincide. Well known examples of fusion partners include, but are not limited to, glutathione-S-transferase (GST), Fc potion of human IgG, maltose binding protein (MBP) and hexahistidine (HIS_6), which are particularly useful for isolation of the fusion polypeptide by affinity chromatography. For the purposes of fusion polypeptide purification by affinity chromatography, relevant matrices for affinity chromatography are glutathione-, amylose-, and nickel- or cobalt-conjugated resins respectively. Many such matrices are available in "kit" form, such as the QIAexpress™ system (Qiagen) useful with (HIS_6) fusion partners and the Pharmacia GST purification system. In a preferred embodiment, the recombinant polynucleotide is expressed in the commercial vector pFLAG as described more fully hereinafter. Another fusion partner well known in the art is green fluorescent protein (GFP). This fusion partner serves as a fluorescent "tag" which allows the fusion polypeptide of the invention to be identified by fluorescence microscopy

or by flow cytometry. The GFP tag is useful when assessing subcellular localisation of the fusion polypeptide of the invention, or for isolating cells which express the fusion polypeptide of the invention. Flow cytometric methods such as fluorescence activated cell sorting (FACS) are particularly useful in this latter application. Preferably, the fusion partners also have protease cleavage sites, such as for Factor X_a or Thrombin, which allow the relevant protease to partially digest the fusion polypeptide of the invention and thereby liberate the recombinant polypeptide of the invention therefrom. The liberated polypeptide can then be isolated from the fusion partner by subsequent chromatographic separation. Fusion partners according to the invention also include within their scope "epitope tags", which are usually short peptide sequences for which a specific antibody is available. Well known examples of epitope tags for which specific monoclonal antibodies are readily available include c-Myc, influenza virus, haemagglutinin and FLAG tags.

Preferred host cells for purposes of selecting vector components for expression vectors of the present invention include fungal host cells such as yeast and prokaryotic host cells such as *E. coli* and *X. albilineans*, but mammalian cell cultures can also be used. In hosts such as yeasts, plants, or mammalian cells that ordinarily do not produce modular polyketide synthase enzymes, it may be necessary to provide, also typically by recombinant means, suitable holo-ACP synthases to convert the recombinantly produced PKS to functionality.

The expression vector may be used to transform the desired host cell to produce a recombinant host cell for producing *inter alia* a recombinant polypeptide or polyketides, particularly albicidins or analogues thereof, as described hereinafter.

5. *Methods of preparing the polypeptides of the invention*

Polypeptides of the inventions, including the full-length parent polypeptides described in Section 2.1, or their biologically active fragments comprising, for example one or more domains (or fragments of such domains), or variants or derivatives of these, may be prepared by any suitable procedure known to those of skill in the art. For example, the polypeptides may be prepared by a procedure including the steps of: -

(a) preparing a recombinant polynucleotide comprising a nucleotide sequence encoding a polypeptide comprising the sequence set forth in any one of SEQ ID NO: 4 or a fragment thereof comprising at least one sequence selected from the group

consisting of SEQ ID NO: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 83, 87, 89, 91, 93, 95, 99, 101, 103, 105 and 107, or variant or derivative of these, which nucleotide sequence is operably linked to a regulatory polynucleotide;

(b) introducing the recombinant polynucleotide into a suitable host cell;

(c) culturing the host cell to express recombinant polypeptide from said recombinant polynucleotide; and

(d) isolating the recombinant polypeptide.

Suitably, said nucleotide sequence comprises at least one sequence selected from the group consisting of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102 and 104.

The recombinant polynucleotide is preferably in the form of an expression vector, which includes a self-replicating extra-chromosomal vector such as a plasmid, or a vector that integrates into a host genome, as for example described above in Section 4. The step of introducing the recombinant polynucleotide into the host cell may be effected by any suitable means including transfection, and transformation, the choice of which will be dependent on the host cell employed. Such methods are well known to those of skill in the art.

Recombinant polypeptides of the invention may be produced by culturing a host cell transformed with an expression vector containing nucleic acid encoding a polypeptide, biologically active fragment, variant or derivative according to the invention. The conditions appropriate for protein expression will vary with the choice of expression vector and the host cell. This is easily ascertained by one skilled in the art through routine experimentation.

Suitable host cells for expression may be prokaryotic or eukaryotic. One preferred host cell for expression of a polypeptide according to the invention is a bacterium. The bacterium used may be *Escherichia coli*. Alternatively, the host cell may be an insect cell such as, for example, *SF9* cells that may be utilised with a baculovirus expression system.

The recombinant protein may be conveniently prepared by a person skilled in the art using standard protocols as for example described in Sambrook, *et al.*, MOLECULAR CLONING. A LABORATORY MANUAL (Cold Spring Harbor Press, 1989), in particular Sections 16 and 17; Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (John Wiley & Sons, Inc. 1994-1998), in particular Chapters 10 and 16; and Coligan *et al.*, CURRENT PROTOCOLS IN PROTEIN SCIENCE (John Wiley & Sons, Inc. 1995-1997), in particular Chapters 1, 5 and 6.

Alternatively, the polypeptide, fragments, variants or derivatives of the invention may be synthesised using solution synthesis or solid phase synthesis as described, for example, in Chapter 9 of Atherton and Shephard (*supra*) and in Roberge *et al* (1995, *Science* **269**: 202).

6. Antigen-binding molecules

The invention also contemplates antigen-binding molecules that bind specifically to the aforementioned polypeptides, fragments, variants and derivatives. Preferably, an antigen-binding molecule according to the invention is immuno-interactive with any one or more of the amino acid sequences set forth in SEQ ID NO: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 83, 87, 89, 91, 93, 95, 99, 101, 103, 105 and 107, or variants thereof.

For example, the antigen-binding molecules may comprise whole polyclonal antibodies. Such antibodies may be prepared, for example, by injecting a polypeptide, fragment, variant or derivative of the invention into a production species, which may include mice or rabbits, to obtain polyclonal antisera. Methods of producing polyclonal antibodies are well known to those skilled in the art. Exemplary protocols which may be used are described for example in Coligan *et al.*, CURRENT PROTOCOLS IN IMMUNOLOGY, (John Wiley & Sons, Inc, 1991), and Ausubel *et al.*, (1994-1998, *supra*), in particular Section III of Chapter 11.

In lieu of the polyclonal antisera obtained in the production species, monoclonal antibodies may be produced using the standard method as described, for example, by Köhler and Milstein (1975, *Nature* **256**, 495-497), or by more recent modifications thereof as described, for example, in Coligan *et al.*, (1991, *supra*) by immortalising spleen or other

antibody producing cells derived from a production species which has been inoculated with one or more of the polypeptides, fragments, variants or derivatives of the invention.

The invention also contemplates as antigen-binding molecules Fv, Fab, Fab' and $F(ab')_2$ immunoglobulin fragments. Alternatively, the antigen-binding molecule may be in the form of a synthetic stabilised Fv (scFv) fragment, a disulphide stabilised Fv (dsFv) fragment, a diabody (dAb), a minibody and the like, or may comprise non-immunoglobulin derived, protein frameworks. The antigen-binding molecules of the invention may be used for affinity chromatography in isolating a natural or recombinant polypeptide or biologically active fragment of the invention. For example reference may be made to immunoaffinity chromatographic procedures described in Chapter 9.5 of Coligan *et al.*, (1995-1997, *supra*). The antigen-binding molecules can be used to screen expression libraries for variant polypeptides of the invention as described herein. They can also be used to detect polypeptides, fragments, variants and derivatives of the invention as described hereinafter.

7. ***Identification of modulators***

The invention also contemplates a method of screening for an agent that modulates the expression of a gene selected from *xabB*, *xabA*, or *xabC*, or a gene belonging to the same regulatory or biosynthetic pathway as *xabB*, *xabA*, or *xabC*, or a variant of that gene, or that modulates the level and/or functional activity of an expression product of that gene or its variant. The method comprises contacting a preparation comprising said expression product (*e.g.*, polypeptide or transcript), or a biologically active fragment thereof, or variant or derivative of these, or a genetic sequence that modulates the expression of said gene (*e.g.*, the natural promoter relating to said gene, *e.g.*, the *xabB* promoter, comprising the sequence set forth in SEQ ID NO: 81 or complement thereof), with a test agent, and detecting a change in the level and/or functional activity of said polypeptide or biologically active fragment thereof, or variant or derivative, or of a product expressed from said genetic sequence.

Modulators contemplated by the present invention includes agonists and antagonists of gene expression include antisense molecules, ribozymes and co-suppression molecules, as for example described in Section 2. Agonists include molecules which increase promoter activity or interfere with negative mechanisms. Agonists of a gene

include molecules which overcome any negative regulatory mechanism. Antagonists of polypeptides encoded by a gene of interest include antibodies and inhibitor peptide fragments.

Candidate agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 Dalton. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including, but not limited to: peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogues or combinations thereof.

Small (non-peptide) molecule modulators of a polypeptide according to the invention, or portion, or domain or module thereof are particularly preferred. In his regard, small organic molecules typically have the ability to gain entry into an appropriate cell and affect the expression of a gene (*e.g.*, by interacting with the regulatory region or transcription factors involved in gene expression); or affect the activity of a gene by inhibiting or enhancing the binding of accessory molecules.

Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc. to produce structural analogues. Screening may also be directed to known pharmacologically active compounds and chemical analogues thereof.

Screening for modulatory agents according to the invention can be achieved by any suitable method. For example, the method may include contacting a cell comprising a polynucleotide corresponding to a gene as defined above, with an agent suspected of having said modulatory activity and screening for the modulation of the level and/or

functional activity of a protein encoded by said polynucleotide, or the modulation of the level of an expression product encoded by the polynucleotide, or the modulation of the activity or expression of a downstream cellular target of said protein or said expression product. Detecting such modulation can be achieved utilising techniques including, but not restricted to, ELISA, cell-based ELISA, filter-binding ELISA, inhibition ELISA, Western blots, immunoprecipitation, slot or dot blot assays, immunostaining, RIA, scintillation proximity assays, fluorescent immunoassays using antigen-binding molecule conjugates or antigen conjugates of fluorescent substances such as fluorescein or rhodamine, Ouchterlony double diffusion analysis, immunoassays employing an avidin-biotin or a streptavidin-biotin detection system, and nucleic acid detection assays including reverse transcriptase polymerase chain reaction (RT-PCR).

It will be understood that a polynucleotide from which a target molecule of interest is regulated or expressed may be naturally occurring in the cell which is the subject of testing or it may have been introduced into the host cell for the purpose of testing. Further, the naturally-occurring or introduced sequence may be constitutively expressed – thereby providing a model useful in screening for agents which down-regulate expression of an encoded product of the sequence wherein said down regulation can be at the nucleic acid or expression product level – or may require activation – thereby providing a model useful in screening for agents that up-regulate expression of an encoded product of the sequence. Further, to the extent that a polynucleotide is introduced into a cell, that polynucleotide may comprise the entire coding sequence which codes for a target polypeptide or it may comprise a portion of that coding sequence (*e.g.* a domain or module as herein described) or a portion that regulates expression of a product encoded by the polynucleotide (*e.g.*, a promoter). For example, the promoter that is naturally associated with the polynucleotide (*ie.* the *xabB* promoter) may be introduced into the cell that is the subject of testing. In this regard, where only the promoter is utilised, detecting modulation of the promoter activity can be achieved, for example, by operably linking the promoter to a suitable reporter polynucleotide including, but not restricted to, green fluorescent protein (GFP), luciferase, ß-galactosidase and catecholamine acetyl transferase (CAT). Modulation of expression may be determined by measuring the activity associated with the reporter polynucleotide.

In another example, the subject of detection could be a downstream regulatory or biosynthetic target of the target molecule, rather than target molecule itself or the reporter molecule operably linked to a promoter of a gene encoding a product the expression of which is regulated by the target protein.

These methods provide a mechanism for performing high throughput screening of putative modulatory agents such as proteinaceous or non-proteinaceous agents comprising synthetic, combinatorial, chemical and natural libraries. These methods will also facilitate the detection of agents which bind either the polynucleotide encoding the target molecule or which modulate the expression of an upstream molecule, which subsequently modulates the expression of the polynucleotide encoding the target molecule. Accordingly, these methods provide a mechanism of detecting agents that either directly or indirectly modulate the expression and/or activity of a gene or expression product according to the invention.

8. Production of secondary metabolites

The present invention further relates to a process for enhancing the level and/or functional activity of secondary metabolites, preferably albicidins, using one or more agents selected from the polynucleotides, polypeptides, fragments, variants, derivatives, vectors and modulatory agents described above. The process in a preferred embodiment, includes the steps of stably transforming a host cell with an expression vector as broadly described above, comprising at least one nucleic acid sequence encoding a polypeptide of the invention or a biologically active fragment or variant or derivative of these and isolating transformants which produce an enhanced amount of antibiotics, which are preferably of the albicidin class. The vector optionally comprises a signal sequence for secretion recognised by the host cell. Illustrative secretory leaders include the secretory leaders of penicillinase, α-factor, immunoglobulin, T-cell receptors, outer membrane proteins, glucoamylase, fungal amylase and the like. By fusion in proper reading frame, the mature polypeptide may be secreted into the medium. The host cell may be a eukaryote or a prokaryote cell. In one embodiment, the cell naturally produces polyketides, preferably antibiotic polyketides and, in this regard, the cell is preferably *X. albilineans* or other bacteria capable of producing albicidins. Optionally, the construct may include a transcription regulating sequence, which is not subject to repression by substances present

in the growth medium. The above process may be used to prepare antibiotics directly or they may be used to prepare cell free extracts containing increased quantities of antibiotics, preferably of the albicidin class, for *in vitro* preparation of said antibiotics. Suitably, these cell free extracts may be prepared for example using the method disclosed by Dobrogosz, W.J. (1981) Enzymatic activity. *In* Manual of Methods for General Bacteriology (Gerhardt, P., ed) Washington, DC: American Society for Microbiology, pp. 365-392. In a preferred embodiment, a vector from which a phosphopantetheinyl transferase (PPTase) can be translated is also introduced into the host cell. Expression of PPTase polynucleotides has been shown to be important for the production of polyketides in heterologous expression systems. Preferably, the PPTase is selected from EntD and/or XabA as for example disclosed herein. If desired, a vector from which a methyltransferase, more preferably and *O*-methyltransferase, and even more preferably an *S*-adenosylmethionine *O*-methyltransferase can be translated may also be introduced into the host cell. An exemplary methyltransferase for this purpose is XabC as described herein.

Alternatively, the expression hosts may be used as a source of increased quantities of antibiotics, which can be subsequently purified as for example disclosed by Birch *et al.* in U.S. Patent No. 4,525,354.

The invention also contemplates use of the polynucleotides, polypeptides, fragments, variant and derivatives of the invention in methods of combinatorial biosynthesis of novel antibiotics as for example disclosed by Khosla *et al.* in U.S. Patent No. 5,712,146, Peterson *et al.* in U.S. Patent No. 5,783,431 and Betlach *et al.* in U.S. Patent No. 6,251,636 or in methods of producing antibiotics in hosts that ordinarily do not produce them as for example disclosed by Barr *et al.* in U.S. Patent No. 6,033,883. As discussed in Section 2.4, the invention contemplates albicidin PKS-NRPS derivatives with altered activities in one or more respects for the production of polyketides other than the albicidin natural product(s) of the XabB. In this regard, expression vectors containing nucleotide sequences encoding a variety of such derivatives for the production of different polyketides are transformed into the appropriate host cells to construct a library. In one embodiment, a mixture of such vectors is transformed into selected host cells and the resulting cells plated into individual colonies and selected to identify successful transformants. A variety of strategies is available to obtain a multiplicity of colonies each containing a PKS gene cluster derived from the naturally occurring host gene cluster so

that each colony in the library produces a different PKS and ultimately a different polyketide, as for example disclosed by Betlach *et al.* in U.S. Patent No. 6,251,636. The libraries thus produced can be considered at four levels: (1) a multiplicity of colonies each with a different PKS-NRPS encoding sequence; (2) the proteins produced from the coding sequences; (3) the polyketides produced from the proteins assembled into a functional PKS-NRPS; and (4) antibiotics or compounds with other desired activities derived from the polyketides. Colonies in the library can be induced to produce the relevant synthases and thus to produce the relevant polyketides to obtain a library of polyketides. Polyketides that are secreted into the media or have been otherwise isolated can be screened for binding to desired targets, such as receptors, signalling proteins, and the like. The supernatants *per se* can be used for screening, or partial or complete purification of the polyketides can first be effected. Typically, such screening methods involve detecting the binding of each member of the library to receptor or other target ligand. Binding can be detected either directly or through a competition assay. Means to screen such libraries for binding are well known in the art. Alternatively, individual polyketide members of the library can be tested against a desired target. In this event, screens wherein the biological response of the target is measured can more readily be included. Antibiotic activity can be verified using typical screening assays such as those for albicidin set forth in Example 1.

The invention also extends to the use of the polynucleotides, polypeptides, fragments, variant and derivatives of the invention for the synthesis of antibiotics, preferably antibiotics of the albicidin class.

The polynucleotides of the invention encoding XabB, or a biologically-active fragment or variant thereof, together with a recombinant polynucleotide encoding a PPTase and/or an *O*-methyltransferase which participate or which are capable of participating in the albicidin biosynthetic pathway, provide the means to engineer high level co-expression of the albicidin synthetase, its activating PPTase and modifying methyltransferase to obtain higher yields of albicidins.

In order that the invention may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting examples.

EXAMPLES

EXAMPLE 1

Albicidin multifunctional synthase gene

Materials and Methods

Bacterial strains and plasmids

The properties of bacteria and plasmids used in this example are listed in Table 1.

Media, culture conditions and antibiotics

X. albilineans strains were routinely cultured on SP medium (Birch & Patil, 1985b) at 28° C. *Escherichia coli* DH5α and JM109 were used as hosts in cloning experiments and were grown on LB medium at 37° C (Sambrook *et al.*, 1989). Broth cultures were aerated by shaking at 200 r.p.m. on an orbital shaker. Modified YEB medium (Van Larebeke *et al.*, 1977) for patch mating consisted of 10 mg ml^{-1} peptone, 5 mg mL^{-1} yeast extract, 5 mg mL^{-1} NaCl, 5 mg mL^{-1} sucrose and 0.5 mg mL^{-1} $MgSO_4.7H_2O$. The following antibiotics were added to media as required: 50 μg kanamycin mL^{-1}; 15 μg tetracycline mLl^{-1}; 100 μg ampicillin mL^{-1}.

Routine genetic procedures

Bacterial genomic DNA and plasmid DNA isolation, gel electrophoresis, DNA restriction digests, ligation reactions and transformation were performed by routine procedures (Sambrook *et al.*, 1989). DNA fragments were excised from agarose gels and residual agarose was removed with the BRESAclean™ DNA purification kit (GeneWorks, Adelaide).

Construction of a X. albilineans partial genomic library

Genomic DNA from *X. albilineans Xa13* was digested with *Eco*RI and size-fractionated. DNA fragments of 15 to 20 kb were ligated to dephosphorylated *Eco*RI-cleaved pBluescript SK II. The ligated DNA was electroporated into E. coli TOP10.

Transformants were selected on LB agar medium containing ampicillin, and stored in LB broth with 15% glycerol at -70°C.

PCR amplification

*Bam*HI-digested genomic DNA from *X. albilineans* LS157 was religated at low concentration (0.5 µg/mL) to generate circular DNA molecules as templates for inverse PCR. Three primers, one from the IS terminal region of Tn5 (IR2: 5'-CGGGATCCTCACATGGAAG TCAGATCCTG-3'), and two flanking the unique BamHI restriction site of Tn5 (BL: 5'-GGGGACCTTGCACAGATAGC-3', and BR: 5'-CATTCCTGTAGCGGATGGAGATC-3'), were used to amplify the sequences flanking the Tn5 insertion in the genome of LS157. The amplified fragments (1.4-kb and 6.0-kb) were cloned into pZErO-2, yielding pZIL and pZIR (Figure 1).

PCR was performed in a volume of 50 µl with 200 ng of genomic DNA (or 10 ng of plasmid DNA), 0.4 ng/µL of each of primer, 0.2 mM of each dNTP, 1.8 mM Mg^{2+}, and 1 unit of elongase enzyme mix (Life Technologies). A 10-min initial denaturation step at 94° C was followed by 35 thermal cycles of denaturation at 94° C for 1 min, annealing at 55° C for 1 min, and extension at 72° C for 1 min per 1 kb of expected amplification.

Construction of promoter probes and glucuronidase assay

Plasmid pRG960sd contains a promoterless β-glucuronidase gene (*uidA*) downstream of a multiple cloning site (Van den Edde *et al.*, 1992). Sequence upstream of xabB (nucleotide residues 1005 to 1210 or 521 to 1210) was amplified from pLXABB by PCR. Forward primer P1F1 (5'-ACGCGGATCCCAGCAGGGTGTCATACACG-3'), or P1F2 (5'-TCGCGGATCC GCGCGATTGAAGTAGTCC-3') contained a BamHI restriction site (underlined). Reverse primer P1R (5'-TCCCCCGGGCGGCCAGCGTGGTGCTACTAC-3') introduced a *Xma*I restriction site (underlined). PCR fragments were ligated into *Bam*HI/*Xma*I-cut pRG960sd, yielding pRG960p1 and pRG960p2. These constructs were mobilised from E. coli DH5α into X. albilineans LS155 as described below.

Promoter strength was quantified by fluorometric analysis of glucuronidase activity (Jefferson, 1987; Xiao *et al.*, 1992). The protein content in cell lysates was determined by the dye-binding method (Bradford, 1976) using a Bio-Rad protein assay kit.

Bacterial conjugation

DNA transfer between *E. coli* donor (JM109 pLAFR3 ± insert, or DH5α pRG960sd ± insert) and *X. albilineans* recipient (LS157 or LS155) was accomplished by triparental transconjugation with helper strain pRK2013. Mid-log-phase cultures of the recipient were spotted onto agar plates containing YEB medium with no antibiotics (20 µL per spot). After the liquid was absorbed by the agar, 20 µL of mid-log-phase culture of the helper was added to each spot. The liquid was again allowed to absorb, and 20 µL of mid-log-phase culture of the donor was added to each spot. After incubation of the mating plates overnight at 28° C, transconjugants were selected on SP plates supplemented with ampicillin, and tetracycline or spectinomycin.

Assay and quantification of albicidin production

Albicidin was quantified by a microbial plate bioassay as described previously (Birch and Patil, 1985b), except that the 10 mL basal layer of LB agar and the 5mL overlayer of 50% LB with 1% agar were supplemented with tetracycline or spectinomycin, and E. coli DH5α pLAFR3 or pRG960sd was used as the indicator strain. This change avoided interference by tetracycline or spectinomycin, which were added to some cultures to ensure retention of pLAFR3 or pRG960sd derivatives in *X. albilineans*. Inhibition zone widths in the bioassay were converted to albicidin concentrations by interpolation on a dose-response plot produced under the same assay conditions. The plot fits the formula: Log [Alb] = 0.3 W – 0.92, where [Alb] is units of albicidin per 20 µL sample assayed, and W is the width in millimetres of the zone of growth inhibition surrounding each well.

Results

Cloning and sequencing of *xabB* gene required for albicidin production

Xanthomonas albilineans Tox$^-$ mutant LS157 contains a single Tn5 insertion, in a 4.1 kb *Cla*I restriction fragment or a 16.5 kb *Eco*RI restriction fragment (Figure 1).

Selection for kanamycin resistance, following shotgun cloning of *Cla*I restriction fragments of LS157 DNA into pBluescript II SK, yielded clone pBC157. Sequences flanking the Tn5 insertion in LS157 DNA were amplified by inverse PCR, and cloned into pZErO-2, producing pZIL and pZIR. Plasmid pLXABB was screened from *a X. albilineans Xa13 Eco*RI genomic library with probes described in Figure 1B. Subclones pSEBL and pSEBR were derived from pLXABB (Figure 1C, Table 1).

The double-strand sequence of the16,511 bp *Eco*RI genomic fragment in pLXABB was obtained by a primer-walking approach, using subclones pBC157, pZIL, pZIR, pSEBL, and pSEBR. The Tn5 insertion in the genome of LS157 is accompanied by 9-bp perfect repeat sequence (GTCCTGAAG), commencing at 2490 bp in GenBank accession no. AF239749.

The only ORF longer than 900 bp within the 16.5-kb fragment is disrupted by the Tn5 insertion. This ORF (designated xabB) encodes a protein of 4081 aa (Mr 525,695). It commences at 1230 bp in GenBank accession no. AF239749 with a TTG codon, 6 bp downstream from a ribosome binding sequence (RBS) GAGG, which may impose post-transcriptional control on the rate of gene product formation (McCarthy and Gualerzi, 1990). There is an alternative start codon (ATG) a further 15 bp downstream. Of the codons in this ORF, 8.5% are rarely used in E. coli. The closest match (TTGAGC-14x-TATAAC) to the consensus -35 (TTGACA) and -10 (TATAAT) sequences for *E. coli* σ^{70} promoters occurs 117 bp upstream of the translation initiation codon (Figure 2).

Downstream by 35 bp from the TAG stop codon of xabB is a probable RBS (GAGG), separated by 6 bp from the ATG start codon of another ORF (designated *xabC*) in the same orientation as *xabB*. Overlapping the *xabB* promoter region is another probable promoter for a divergent transcript including a putative RBS (TGGAGG) and start codon for a gene designated xatA, separated by 233 bp from *xabB* (Figure 1, 2).

Complementation of *xabB* gene in LS157

Mobilisation of pLAFR3, pLXABB1 or pLXABB2 by bacterial conjugation into Tox$^-$ mutant LS157 occurred at a frequency of 1.5×10^{-2} transconjugants/recipient cells. Albicidin production was undetectable in Tox$^-$ mutant LS157 and LS157 (pLAFR3)

controls, but introduction of the *xabB* gene on pLXABB1 or pLXABB2 restored albicidin production to the level of the wild-type parental strain LS155 (Figure 4).

Functional analysis of *xabB* promoter region

GUS activity was undetectable in LS155 and LS155 (pRG960sd) controls. Plasmid pRG960p1 or pRG960p2, with 206 bp or 690 bp from the *xabB* promoter region upstream of GUS, both conferred GUS activity with no difference in expression level or pattern in *X. albilineans* LS155 (Figure 5).

Discussion

Albicidin was partially characterised as a low-molecular-weight compound that contains 38 carbon atoms with 3-4 aromatic rings (Birch and Patil, 1985a). The compound is not degraded by peptidases (Birch and Patil, 1985a), but it is cleaved by the AlbD esterase (Zhang and Birch, 1997). Based on the deduced functionality of the synthase describe herein, albicidin is likely to be a complex polyketide, condensed with amino acid(s), or nonproteinogenic amino, hydroxyl and carboxyl acid(s) by C-N, amide or ester bond formation.

The characterisation of XabB as a multi-modular hybrid enzyme provides new insights into the mechanism of albicidin biosynthesis and possible approaches to engineer the overproduction of albicidins. For example, the complementation experiments (Figure 4) indicate that increased copy number of *xabB* stimulates early production of albicidin, but other factors become limiting during idiophase. It may be possible to increase expression of the albicidin synthase by modifications to the promoter and TTG start codon, or to improve albicidin yields by supplying candidate substrates (such as shikimate-derived units). The unusual enzyme organisation also contributes to the emerging understanding of how microbes generate structural diversity of antibiotics, and can facilitate combinatorial engineering of antibiotics of mixed peptide/polyketide origin.

EXAMPLE 2

Albicidin Antibiotic and Phytotoxin Biosynthesis in Xanthomonas albilineans *Requires a Phosphopantetheinyl Transferase Gene*

Materials and Methods

Bacterial strains and plasmids

The properties of bacteria and plasmids used in this Example are listed in Table 3.

Media, culture conditions and antibiotics

X. albilineans strains were routinely cultured on SP medium (Birch & Patil, 1985b) at 28° C. *Escherichia coli* DH5α and JM109 were used as hosts in cloning experiments and were grown on LB medium at 37° C (Sambrook *et al.*, 1989). Broth cultures were aerated by shaking at 200 r.p.m. on an orbital shaker. Modified YEB medium (Van Larebeke *et al.*, 1977) for patch mating consisted of 10 mg ml^{-1} peptone, 5 mg mL^{-1} yeast extract, 5 mg mL^{-1} NaCl, 5 mg mL^{-1} sucrose and 0.5 mg mL^{-1} $MgSO_4.7H_2O$. The following antibiotics were added to media as required: 50 μg kanamycin mL^{-1}; 15 μg tetracycline mL^{-1}, 100 μg ampicillin mL^{-1}.

Assay of albicidin production

Albicidin was quantified by a microbial plate bioassay as described previously (Birch and Patil, 1985b), except that the 10 mL basal layer of LB agar and the 5 mL overlayer of 50% LB with 1% agar were supplemented with tetracycline, and E. coli DH5α [pLAFR3] was used as the indicator strain. This change avoided interference by tetracycline, which was added to some cultures to ensure retention of pLAFR3 derivatives in *X. albilineans*.

Routine genetic procedures

Bacterial genomic DNA and plasmid DNA isolation, gel electrophoresis, DNA restriction digests, ligation reactions and transformation were performed by routine procedures (Sambrook *et al.*, 1989). DNA fragments were excised from agarose gels and

residual agarose was removed with the BRESAclean™ DNA purification kit (GeneWorks, Adelaide).

DNA sequencing and analysis

Sequencing reactions were performed by dideoxynucleotide chain termination (Sanger *et al.*, 1977) using the BigDye™ Terminator Cycle Sequencing Kit and 373A DNA sequencer (PE Applied Biosystems) through the Australian Genome Research Facility. Oligonucleotide primers were purchased from GeneWorks (Adelaide). University of Wisconsin Genetics Computer Group (UWGCG) programs BLASTP, FASTA, PILEUP, and BESTFIT were used through WebANGIS version 2.0 for DNA and protein sequence analyses of the GenBank, EMBL, PIR and SWISSPROT databases using standard defaults.

Cloning of Tn5 flanking sequences

*Eco*RI-digested genomic DNA from *X. albilineans* Tox⁻ mutant LS156 was ligated into pBluescript II SK and electroporated into *E. coli* DH5α. Transformants were selected on LB medium containing kanamycin and ampicillin, yielding clone pBEA1, from which subclones pCEA1 and pPEA1 were obtained (Figure 1).

Amplification of sequences from wild-type LS155 by PCR

Sequences flanking the Tn5 insertion in LS156 were used to design primers (A1F: 5'-TTTGGGTTGGATCGGGTAG-3' and A1R: 5'-CCTTCTCGTCCTTG CTCTTC-3') for PCR-amplification of the corresponding wild type *X. albilineans* LS155 chromosomal DNA. PCR was performed in a volume of 50 µL with 200 ng of genomic DNA, 0.4 ng μL^{-1} of each of primer, 0.2 mM of each of dNTP, 1.8 mM Mg^{2+}, and 1 unit of elongase enzyme mix (Life Technologies). A 4-min initial denaturation step at 94° C was followed by 35 thermal cycles of denaturation at 94° C for 1 min, annealing at 55° C for 1 min, and extension at 72° C for 2 min. The amplified DNA fragment was cloned into pGEM-T to give pGTA1 (Figure 1).

Construction of expression vectors

The coding region of the *xabA* gene was amplified from pGTA1 by PCR. Primer A1F1 (5'-GGAATTCCATGCCCAATGCCGTACCG-3') contained an *Eco*RI restriction

site (underlined) for insertion of the amplified gene into the correct reading frame of *lacZ* in pLAFR3. Primer A1R1 (5'-CGGGATCCCGTGCTCACCAGGCGTAGTGG-3') introduced a *Bam*HI restriction site (underlined), 5 bases downstream from the stop codon of the amplified gene. The amplified DNA fragment was digested with *Eco*RI and *Bam*HI, and ligated with *Eco*RI/*Bam*HI-digested pLAFR3 to result in pLXABA.

Similarly, the coding region of the *entD* gene was PCR-amplified from *E. coli* DH5α by colony PCR using primers EntDF (5'-TCCCGGAATTCCATGGTCGATATGAAAACTACGC-3') and EntDR (5'-GCCCAAGCTTCTAATCGTGTTGGCACAGCGTTATG-3'), then ligated into pLAFR3 to produce pLENTD. The inserts in pLXABA and pLENTD were sequenced to confirm the expected clones.

Bacterial triparental mating

DNA transfer between *E. coli* donor (JM109 pLAFR3 ± insert) and *X. albilineans* recipient (LS155 or LS156) was accomplished by triparental transconjugation with helper strain pRK2013. The mid-log-phase cultures of the recipient were spotted onto agar plates containing YEB medium with no antibiotics (20 µL per spot). After the liquid was absorbed by the agar, 20 µL of mid-log-phase culture of the helper was added to each spot. The liquid was again allowed to absorb, and 20 µl of mid-log-phase culture of the donor was added to each spot. After incubation of the mating plates overnight at 28° C, transconjugants were selected on SP plates supplemented with tetracycline and ampicillin.

Results

Cloning and sequencing of the xabA gene required for albicidin production

Xanthomonas albilineans Tox⁻ mutant LS156 contains a single Tn5 insertion, in a 3.0-kb *Eco*RI restriction fragment (Wall & Birch, 1997). Selection for Tn5-encoded kanamycin resistance, following shotgun cloning of *Eco*RI restriction fragments of LS156 DNA into pBluescript II SK, yielded pBEA1 (Figure 8).

Both strands of the insert in pBEA1 excluding the Tn5 insertion were sequenced by primer-walking from T3 and T7 vector sequences in pBEA1 and subclones pCEA1 and

pPEA1. The corresponding genomic region was amplified from wild-type *X. albilineans* LS155 by PCR, and cloned into pGEM-T to give pGTA1. Sequencing of pGTA1 revealed that a 9-bp imperfect repeat sequence (TTGGCCACG) in the genome of LS156 accompanied the Tn5 insertion (following base number 1869 in Figure 9). The double-strand nucleotide sequence of the 2989 bp wild type *Eco*RI fragment is deposited in GenBank under accession no. AF191324.

Reading frame analysis of the 3 kb *Eco*RI fragment revealed that only one ORF (designated xabA) is disrupted by the Tn5 insertion. This ORF encodes a protein of 278 aa (Mr 29 277), with 6.12% codons rarely used in *E. coli*. There were no close matches to *E. coli* -10 (TATAAT) and -35 (TTGACA) consensus promoter sequences, and no appropriately spaced RBS sequence (such as AGGA or GAGG) in the region upstream of the putative start codon ATG (Figure 9). A region of GC-rich dyad symmetry with a free energy of –10.2 kcals/mol was found, followed by two TCTC boxes that closely resemble the TCTG consensus sequence characteristic of many factor-independent termination sites (Brendel & Trifonov, 1984; Platt, 1986) downstream of the TGA termination codon of xabA.

Comparison of XabA with other bacterial PPTases

A search for proteins with homology to the deduced *xabA* product, using the FASTA and BLASTP and SWISSPROT programs, indicated regions of similarity to EntD from *Escherichia coli* (170 aa overlap, 35.9 % identity, 56.5 % similarity), *Shigella flexneri* (180 aa overlap, 35.0 % identity, 55.6 % similarity), *Salmonella typhimurium* (184 aa overlap, 35.9 % identity, 62.0 % similarity), and *Salmonella austin* (172 aa overlap, 36.1 % identity, 61.1 % similarity). XabA contains (V/I)G(V/I)D and (F/W)(S/C/T)xKE(S/A)xxK domains characteristic of the phosphopantetheinyl transferase (PPTase) superfamily, and shares 17-36 % overall identity, 39-62 % overall similarity, with other bacterial PPTases (Table 4).

Enhanced expression of xabA by complementation in LS156 results in increased production of albicidins

Mobilisation of pLAFR3 or pLXABA (pLAFR3::*xabA*) by triparental matings into Tox$^-$ mutant LS156 occurred at a frequency of 1.5 x 10^{-2} transconjugants/recipient

cells. Albicidin production was undetectable in Tox$^-$ mutant LS156 and LS156 (pLAFR3) controls, but introduction of the *xabA* gene on pLXABA enhanced albicidin production restored albicidin production (Figure 10). In LS156 (pLXABA), as in LS155, albicidin was first detectable in late-log-phase cultures (OD_{550} = 0.7) and was maximal in stationary phase. Albicidin production was not responsive to IPTG or glucose, and the *lac* promoter driving *xabA* in pLXABA is considered to express constitutively in *X. albilineans*. The *E. coli entD* gene, expressed from the *lac* promoter in pLENTD, also complemented the *xabA*::Tn5 mutation, restoring albicidin production in LS156.

Discussion

A gene required for albicidin production in *X. albilineans* was isolated using a Tn5 mutagenesis and shotgun cloning approach. The ORF interrupted by Tn5 in Tox$^-$ mutant LS156 is designated *xabA*. This ORF was isolated from Tox$^+$ parent strain LS155, and shown to enhance albicidin production early in the production phase in LS156 when expressed from the *lac* promoter in pLAFR3. Tn5 insertions typically cause polar mutations affecting all downstream cistrons in an operon (De Bruijn and Lupski, 1984). Complementation of the mutation in LS156 by the isolated *xabA* ORF indicates the absence of any downstream cistron involved in albicidin production. There is no consensus RBS sequence close to the alternative start codons for this ORF in the *X. albilineans* genome. Translation may be initiated without an evident ribosome binding sequence complementary to the 3' end of the 16S rRNA, as observed for some streptomycete genes involved in secondary metabolism (Strohl, 1992), and for some chloroplast genes (Kozak, 1999).

PPTases play an essential role in priming polyketide, fatty acid, non-ribosomal peptide and siderophore biosynthesis (Gehring *et al.*, 1997a; Lambalot *et al.*, 1996; Marahiel *et al.*, 1997; Walsh *et al.*, 1997). All polyketide synthase, fatty acid synthetases, and non-ribosomal peptide synthetases require post-translational modification to become catalytically active (Walsh *et al.*, 1997). The inactive apo-proteins are converted to their active holo-forms by transfer of the 4'-phosphopantetheinyl (P-pant) moiety of coenzyme A to the sidechain hydroxyl of a serine residue in a conserved carrier domain (Lambalot *et al.*, 1996; Walsh *et al.*, 1997). The P-pant moiety serves to covalently tether the growing

product, which is assembled by sequential action of multiple catalytic domains in these complex synthetases (Walsh *et al.*, 1997).

A family of more than twenty PPTases is recognised by a common (V/I)G(V/I)Dx40-45...(F/W)(S/C/T)xKE(A/S)xxK signature sequence, but overall sequence homologies are low (Gehring *et al.*, 1997; Lambalot *et al.*, 1996; Nakano *et al.*, 1992; Quadri *et al.*, 1998a). In *E. coli*, there are two PPTases with distinct specificities: ACPS is active on acyl carrier protein (ACP) domains in fatty acid and polyketide synthase; EntD is active on peptidyl carrier protein (PCP) and aryl carrier protein (ArCP) domains in peptide synthetases (Lambalot *et al.*, 1996; Walsh *et al.*, 1997). Thus, PPTases may be partner-protein specific. However, Sfp from *B. subtilis* appears to be non-specific, efficiently activating both fatty acid, polyketide synthase and peptide synthetases (Kealey *et al.*, 1998; Mofid *et al.*, 1999; Quadri *et al.*, 1998a). XabA includes the PPTase VGID and FSxKESxxK motifs. Although it has highest overall similarity to the peptide-selective EntD proteins, the sequence groupings are not sufficiently compelling to predict the specificity of XabA for polyketide synthase or peptide synthetases (Table 4, Figure 11).

Complementation studies have revealed substantial functional interchangeability of PPTases in different bacteria. For example, the *B. sublitis sfp* gene involved in surfactin biosynthesis complements mutants in *E. coli entD* (enterobactin biosynthesis) and *B. brevis gsp* (gramicidin biosynthesis) (Borchert *et al.*, 1994; Grossman *et al.*, 1993). In vitro, ACPS from *E. coli* activates apoproteins from *Lactobacillus, Rhizobium* and *Streptomyces* (Lambalot *et al.*, 1996). Because XabA shows highest similarity to EntD, we amplified the *entD*-coding region from *E. coli*, and arranged it for expression from the *lac* promoter in broad host-range vector pLAFR3. This construct (pLENTD) restored albicidin production in *X. albilineans xabA*::Tn5 mutant LS156. EntD is a peptide-selective PPTase that converts inactive apo-EntF and apo-EntB to active holo-enzymes involved in biosynthesis of enterobactin in *E. coli* (Gehring *et al.*, 1997a). Functional complementation of the *xabA*::Tn5 mutation by *entD* indicates that XabA is a PPTase required for post-translational activation of synthetases involved in albicidin production in *X. albilineans*. The specificity of EntD for activation of peptide synthetases in *E. coli* indicates that albicidin biosynthesis probably involves an XabA-activated peptide synthetase.

Some PPTase genes involved in non-ribosomally synthesised peptide biogenesis are located near the genes encoding their targets (Quadri *et al.*, 1998b). For example, *B. brevis gsp, B. sublitis sfp,* and *E. coli entD* genes all lie within 4 kb of operons encoding the target peptide synthetases (Borchert *et al.*, 1994; Coderre & Earhart, 1989; Nakano *et al.*, 1992). However, *M. tuberculosis pptT* is not located near the *mbt* gene cluster encoding the target peptide synthetases involved in mycobactin biosynthesis (Quadri *et al.*, 1998b). No gene encoding a PPTase has been identified in any of the antibiotic and phytotoxin biosynthetic gene clusters characterised from *Streptomyces* spp. (Gehring *et al.*, 1997b) and *Pseudomonas* spp. (Bender *et al.*, 1999). No evident target gene was found within 1282 bp upstream or 870 bp downstream of *xabA*. Three cosmids spanning about 100 kb in two regions of the genome complemented 56 of 58 tested Tox$^-$ mutants of *X. albilineans*, but not LS156 (Rott *et al.*, 1996). These results indicate that *xabA* is not clustered with the genes encoding the antibiotic synthetases that it activates.

Expression of *xabA* (or an alternative PPTase such as *entD*) is essential for albicidin biosynthesis. The phosphopantetheinyl transferase gene described herein provides new insight into antibiotic biosynthesis in the *Pseudomonadaceae*, and new opportunities to understand and apply albicidins as potent inhibitors of prokaryote DNA replication. This gene, together with the *xabB* provide the means to engineer high level co-expression of the albicidin synthetase and its activating PPTase to obtain higher yields of albicidins, and ultimately to manipulate the elements of this biosynthetic machinery, by mutagenesis or otherwise, to produce desired structural variants of this novel antibiotic class. They may also indicate a new approach to disease resistance, by engineering plants to interfere with the biosynthesis of albicidin toxins, which are key pathogenesis factors for the systemic development of leaf scald disease.

EXAMPLE 3

A methyltransferase gene is involved in albicidin biosynthesis in Xanthomonas albilineans

Material and Methods

Bacterial strains and plasmids

The properties of bacteria and plasmids used in this example are listed in Table 5.

Media, culture conditions and antibiotics

X. albilineans strains were routinely cultured on sucrose peptone (SP) medium at 28° C (Birch and Patil, 1985b). Escherichia coli strains were used as hosts in cloning experiments and were grown on LB medium at 37° C (Sambrook *et al.*, 1989). Broth cultures were aerated by shaking at 200 rpm on an orbital shaker. Modified YEB medium (Van Larebere *et al.*, 1977) was used for patch mating. The following antibiotics were added to media as required: kanamycin, 50 µg/mL; tetracycline, 15 µg/mL; ampicillin, 100 µg/mL.

Assay of albicidin production

Albicidin was quantified by a microbial plate bioassay as described previously (Birch and Patil, 1985b), except that the 10 mL basal layer of LB agar and the 5 mL overlayer of 50% LB with 1% agar were supplemented with tetracycline, and *E. coli* DH5α [pLAFR3] was used as the indicator strain. This change avoided interference by tetracycline, which was added to some cultures to ensure retention of pLAFR3 derivatives in *X. albilineans*.

Routine genetic procedures

Bacterial genomic DNA and plasmid DNA isolation, gel electrophoresis, DNA restriction digests, ligation reactions and transformation were performed by routine procedures (Sambrook *et al.*, 1989). DNA fragments were excised from agarose gels and residual agarose was removed with the BRESAclean™ DNA purification kit (GeneWorks, Adelaide).

DNA sequencing and analysis

Sequencing reactions were performed by dideoxynucleotide chain termination (Sanger *et al.*, 1977) using the BigDye™ Terminator Cycle Sequencing Kit and 373A DNA sequencer (PE Applied Biosystems) through the Australian Genome Research Facility. Oligonucleotide primers were purchased from GeneWorks (Adelaide). University of Wisconsin Genetics Computer Group (UWGCG) programs BLASTP, FASTA, PILEUP, and BESTFIT were used through WebANGIS version 2.0 for DNA and protein sequence analyses of the GenBank, EMBL, PIR and SWISSPROT databases.

Recovery of the downstream sequence of truncated *xabC* by IPCR

Genomic DNA of *X. albilineans* LS155 was digested with *Nco*I. Following phenol/chloroform extraction and ethanol precipitation, the digested DNA was self-ligated at a concentration of 0.5 µg/mL. The ligated DNA was precipitated with ethanol and resuspended in sterile H_2O to a concentration of 20 ng/µL as template for IPCR. Sequence of the 16.5 kb *Eco*RI fragment including the 5' region of *xabC* was used to design primers (IF: 5'-AAGCGTCGACATAGCAGCAG-3' and IR: 5'-CGGCAACGCATTCGACCTCG-3') for IPCR-amplification of the sequence downstream of the *Eco*RI site of truncated *xabC* gene.

IPCR was performed in a volume of 50 µL with 50 ng of template DNA, 0.4 ng/µL of each of primer, 0.2 mM of each of dNTP, 1.8 mM Mg^{2+}, and 1 unit of elongase enzyme mix with proof-reading activity (Life Technologies). A 10 min initial denaturation step at 94° C was followed by 35 thermal cycles of denaturation at 94° C for 1 min, annealing at 55° C for 1 min, and extension at 72° C for 1 min per 1 kb of expected amplification product. The IPCR product was cloned into pZErO-2 to give pZIXC. Clones of construct pZIXC from three independent PCR reactions were sequenced to rule out the possibility of PCR-generated errors.

Insertional mutagenesis

An internal 625 bp *Cla*I-*Eco*RI fragment of *xabC* (Figure 13) was firstly cloned into *ClaI*/*Eco*RI-digested pBluescript II SK to provide a *Kpn*I restriction site, then subcloned into *Eco*RI/*Kpn*I-cleaved pJP5603 to yield pJP-BEC. The inserts in pBluescript II SK intermediates (pBEC) were sequenced to confirm the expected clones.

The suicide construct pJP-BEC was transferred from the mobilising strain E. coli S17-1 (λpir) into *X. albilineans* LS155. Exconjugant colonies were selected on SP agar containing kanamycin and ampicillin. Insertional disruption in xabC or thp was verified by PCR using primers flanking the expected integration site of pJP-BEC or pJP-BAS and extension at 72° C for 1 min as previously described (Zhang and Birch, 1997b). The effect on albicidin biosynthesis was determined using the microbial plate assay. Representative (Tox$^-$) insertional mutants in *xabC* (LS-JP1) and *thp* (LS-JP2) were retained for further analysis.

Construction of expression vectors

The coding region of the *xabC* gene was amplified from *X. albilineans* LS155 chromosomal DNA by PCR. Primer A3F (5'-CGGGATCCCATGGATTCAGCGTTACC-3') contained a BamHI restriction site (underlined) for insertion of the amplified gene into the correct reading frame of *lacZ* in pLAFR3. Primer A3R (5'-CCCAAGCTTTCATTAT GGGGCCCTCTTGC-3') introduced a *Hin*dIII restriction site (underlined). The amplified DNA was digested with *Bam*HI and *Hin*dIII, and ligated with *Bam*HI/*Hin*dIII-digested pLAFR3 to result in pLXABC. *X. albilineans* Tox⁻ mutant LS157 contains a single Tn5 insertion, in a 4.1 kb *Cla*I restriction fragment or a 16.5 kb EcoRI restriction fragment (Figure 12). Selection for kanamycin resistance, following shotgun cloning of *Cla*I restriction fragments of LS157 DNA into pBluescript II SK, yielded clone pBC157. Sequences flanking the Tn5 insertion in LS157 DNA were amplified by inverse PCR, and cloned into pZErO-2, producing pZIL and pZIR. The double-strand sequence of the 16,511 bp *Eco*RI genomic fragment in pLXABB was obtained by a primer-walking approach, using subclones pBC157, pZIL, pZIR, pSEBL, and pSEBR. The Tn5 insertion in the genome of LS157 is accompanied by 9-bp perfect repeat sequence (GTCCTGAAG), commencing at 2490 bp in GenBank accession no. AF239749.

Genetic complementation of albicidin biosynthesis

DNA transfer between E. coli donor (JM109 pLAFR3 ± insert) and *X. albilineans* recipient (LS-JP1 or LS-JP2), was accomplished by triparental transconjugation with helper strain pRK2013. Mid-log-phase cultures of the recipient were spotted onto agar plates containing YEB medium with no antibiotics (20 µL per spot). After the liquid was absorbed by the agar, 20 µL of mid-log-phase culture of the helper was added to each spot. The liquid was again allowed to absorb, and 20 µL of mid-log-phase culture of the donor was added to each spot. After incubation of the mating plates overnight at 28° C, transconjugants were selected on SP plates supplemented with ampicillin, and tetracycline or spectinomycin.

Transconjugants were tested for albicidin production using the microbial plate bioassay. The constructs pLXABB, pLXABC were designed to test complementation in trans. However, complementation could also occur in *cis*, by homologous recombination between the complementing construct and the insertionally mutated chromosomal gene. To

exclude this possibility, the retention of the insertion in *xabC* was confirmed by PCR, using primers from *aphA* (in the insertion) and *xabB* (adjoining *xabC* in the chromosome).

Results and Discussion

Cloning and sequencing of the full-length *xabC* gene

Downstream by 45 bp from the TAG stop codon of *xabB* is the start of an ORF (designated *xabC*) in the same orientation. The 639-bp sequence downstream of the *Eco*RI site of the truncated *xabC* was amplified from wt *X. albilineans* LS155 using IPCR. The double-strand nucleotide sequence of 1515 bp from the stop codon of *xabB* to the *Nco*I site downstream of *xabC* (Figure 13) is deposited in GenBank under accession no. AF239750. The *xabC* ORF encodes a protein of 343 aa (Mr 37,704). One TCTG-like sequence (TGTG) and one typical TCTG box characteristic of many factor independent termination sites (Brendel and Trifonov, 1984) occur downstream of the termination codon (TAA) of xabC (Fig. 2). However, the other features typical of such terminators (a region of GC rich dyad symmetry, followed by a run of consecutive thymine residues) are not present within 435 bp downstream of the *xabC* stop codon.

XabC is similar to O-methyltransferases

The deduced product of xabC shows 22-30% overall identity and 52-60% overall similarity to a family of methyltransferases that utilise S-adenosyl-methionine (SAM) as a co-substrate for O-methylation of small molecules (Ingrosso *et al.*, 1989; Haydock *et al.*, 1991; Kagan and Clarke, 1994). These enzymes include tetracenomycin polyketide C-8 O-methyltransferase (TcmO, P39896) and C-3 O-methyltransferase (TcmN, P16559) of *Streptomyces glaucescens*, hydroxyneurosporene-O-methyltransferase (P17061) of *Rhodobacterium capsulatus*, and hydroxyindole-O-methyltransferases of rat pineal and retina (O09179) and chicken pineal gland (Q92056). Three highly conserved motifs in SAM-dependent methyltransferases are also present in XabC as shown in Figures 13 and 14. The crystal structure analysis for the methyltransferase-SAM complex (Schlukebier *et al.*, 1995) provides firm structural evidence for the role of motif I in SAM binding.

Insertional mutagenesis of *xabC* blocks albicidin biosynthesis

Insertional mutation in *xabC* was accomplished using suicide-vector pJP-BEC and confirmed by PCR. Six out of eight tested transconjugants were verified by PCR to contain insertional mutations in *xabC*. Albicidin production was undetectable in these insertional mutants, compared to wt X. *albilineans* LS155 control. The other transconjugants may result from integration of the vector at other genomic locations by illegitimate recombinations as reported previously (Penfold and Pemberton, 1992).

Complementation test

Introduction of the *xabC* gene in pLXABC or the truncated *xabC* gene in pLXABB into insertional mutant LS-JP2 restored albicidin production to the level of the wt parental strain LS155. This indicates that *xabC* is essential for albicidin production in *X. albilineans*. The truncated *xabC* in pLXABB (SEQ ID NO: 106) encodes 277 residues (SEQ ID NO: 107), including all of the three conserved motifs of SAM-methyltransferases, and appears fully functional by complementation. The continued presence of an insertion in the chromosomal locus was confirmed by PCR. Thus, complementation was operating in *trans*. This also indicates that no other cistron downstream of *xabC* is required for albicidin production, because insertional mutagenesis typically causes polar mutations affecting all downstream cistrons in an operon (De Bruijn and Lupski, 1989).

Enhanced expression of *xabC* results in increased production of albicidins

Derivatives of *X. albilineans* strain LS155, in which an *xabC* gene, or fragment thereof, was introduced in *trans*, were tested for production of albicidin using the bioassay described above. The results, presented in Figure 15, show that expression of *xabC* cloned into pLAFR3 in derivatives of *X. albilineans* strain LS155 complements an insertional mutation in the chromosomal *xabC*, and also enhances albicidin production early in the production phase. Expression of the first part of the *xabB* operon, including the full-length *xabB* and a truncated but functional *xabC*, further enhances albicidin production.

The disclosure of every patent, patent application, and publication cited herein is hereby incorporated herein by reference in its entirety.

The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application

Throughout the specification the aim has been to describe the preferred embodiments of the invention without limiting the invention to any one embodiment or specific collection of features. Those of skill in the art will therefore appreciate that, in light of the instant disclosure, various modifications and changes can be made in the particular embodiments exemplified without departing from the scope of the present invention. All such modifications and changes are intended to be included within the scope of the appended claims.

TABLES

TABLE 1

Bacterial strains, and plasmids for Example 1

Strain or plasmids	*Relevant characteristics*	*Reference or source*
Strains		
E. coli		
DH5α	Φ80dlacZΔM15, Δ(lacZYA-argF	Promega
JM109	[F', lacI^{q}ZΔM15], Δ(lac-proAB	Promega
TOP10	F$^-$, Δ(mrr-hsdRMS-mcrBC), Δ(are-leu)7697, ΔlacX74	Invitrogen
X. albilineans		
Xa13	Wild-type albicidin producer from sugarcane (Queensland), Ap^r	Inventor's laboratory
LS155	Wild-type albicidin producer from sugarcane (Queensland), Ap^r	Wall and Birch (1997)
LS157	LS155::Tn5, albicidin deficient (Tox$^-$), Km^r St^r Ap^r	Wall and Birch (1997)
Plasmids		
pBluescript II SK	ColE1 origin, E. coli cloning vector, Ap^r	Stratagene
pZErO-2	ColE1 origin, E. coli cloning vector, Km^r	Invitrogen
pRK2013	ColE1origin, IncP, Tra$^+$, helper plasmid, Km^r	Ditta et al ('980)
pLAFR3	RK2 origin, Tra$^-$, Mob$^+$, broad host-range cosmid, Tc^r	Stachelhaus at al. (1987)
pRG960sd	ColE1origin, broad host-range plasmid, contains promoterless uidA with start codon and Shine-Dalgarno sequence, Sm^r Sp^r	Van den Edde *et al.* (1992)
pBC157	9.9-kb ClaI fragment carrying Tn5 and flanking sequences from LS157, in pBluescript II SK, Km^r Ap^r	This study
pZIL	1.4-kb fragment, inverse PCR amplified from LS157 in pZErO-2, Km^r	This study
pZIR	6.0-kb fragment, inverse PCR amplified from LS157 in pZErO-2, Km^r	This study
pZTI	0.9-kb fragment, PCR amplified from LS157 in pZErO-2, Km^r	This study
pXABB	16.5-kb EcoRI fragment from Xa13 in pBluescript II SK, Ap^r	This study
pSEBL	7.9-kb EcoRI-SpeI frament from pXABB in pBluescript II SK, Ap^r	This study
pSEBR	8.6-kb EcoRI-SpeI frament from pXABB in pBluescript II SK, Ap^r	This study

Strain or plasmids	*Relevant characteristics*	*Reference or source*
pLXABB1	16.5-kb EcoRI fragment from pXABB in pLAFR3 (xabB in the same direction as lac), Tc^r	This study
pLXABB2	16.5-kb EcoRI fragment from pXABB in pLAFR3 (xabB in the opposite direction to lac), Tc^r	This study
pRG960p1	206-bp BamHI-XmaI frament in pRG960sd, Sm^r Sp^r	This study
pRG960p2	690-bp BamHI-XmaI frament in pRG960sd, Sm^r Sp^r	This study

TABLE 2

Comparison of conserved sequences in peptide synthetases and XabB

Domain	*Core*	*Sequence conserved in peptide synthetases*[a]	*Sequence in XabB*	*Position in Xab (aa)*
Adenylation	A1	L(T/S)YxEL	WSYAQL	3806-3811
	A2	LKAGxAYL(V/L)P(L/I)D	FKAGACYVPID	3851-3861
	A3	LAYxxYTSG(S/T)TGxPKG	LACVMVTSGSTGRPKG	3917-3932
	A4	FDxS	FAVS	3967-3970
	A5	NxYGPTE	NNYGCTE	4063-4069
	A6	GELxIxGxG(V/L)ARGYL	GELHVHSVGMARGYW	4114-4128
	A7	Y(R/K)TGDL	YKTGDM	4152-4157
	A8	GRxDxQVKIRGxRIELGEIE	GRQDFEVKVRGHRVDTRQVE	4170-4189
	A9	LPxYM(I/V)P	LPTYMLP	4239-4245
	A10	NGK(V/L)DR	NGKLDR	4259-4264
Peptidyl carrier protein	PCP	DxFFxLGG(H/D)S(L/I)	DNFFALGGHSL MDFFAVGGHSV	4306-4316 3261-3271
Condensation	C1	SxAQxR(L/M)(W/Y)xL	TYAQERLWLV SLFQERLWFV	3333-3342 4374-4383
	C2	RHExLRTxF	RHEVLRTRF RHEILRTRF	3381-3389 4421-4429
	C3	MHHxISDG(W/V)S	IHHIISDGWS MHHLIYDAWS	3456-3465 4498-4507
	C4	YxD(F/Y)AVW	YADYALW YADYAIW	3495-3501 4538-4544
	C5	(I/V)GxFVNT(Q/L)(C/A)xR	IGFFINILPLR IGFFINILPLR	3606-3617 4649-4659
	C6	(H/N)QD(Y/V)PFE	HQSVPFE NQALPFE	3641-3647 4685-4691
	C7	RDxSRNPL	RDSSQIPL RDTSRIPL	3658-3665 4701-4708

[a]*Sourced from reference (Marahiel et al., 1997).*

TABLE 3

Bacterial strains, and plasmids for Example 2

Strain or plasmids	*Relevant characteristics*	*Reference or source*
Strains		
E. coli		
DH5α	Φ80dlacZΔM15, recA1, endA1, gyrA96, thi-1, hsdR17(r_k^-, m_k^+) supE44, relA1, deoR, Δ(lacZYA-argF)U169	Promega
JM109	[F', traD36, proAB, lacI^{q}ZΔM15], recA1, endA1, gyrA96, thi hsdR17(r_k^-, m_k^+), supE44, relA1, Δ(lac-proAB)	Promega
X. albilineans		
Xa13	Wild-type albicidin producer from sugarcane (Queensland), Ap^r	This laboratory
LS155	Wild-type albicidin producer from sugarcane (Queensland), Ap^r	Wall & Birch (1997)
LS156	LS155::Tn5, albicidin deficient (Tox$^-$), Km^r St^r Ap^r	Wall & Birch (1997)
Plasmids		
pBluescript II SK	ColE1 origin, E. coli cloning vector, Ap^r	Stratagene
pGEM-T	ColE1 origin, E. coli TA-cloning vector, Ap^r	Promega
pRK2013	ColE1origin, IncP, Tra$^+$, helper plasmid, Km^r	Ditta et al. (1980)
pLAFR3	RK2 origin, Tra$^-$, Mob$^+$, broad host-range cosmid, Tc^r	Staskawicz et al. (1987)
pBEA1	8.8-kb EcoRI fragment carrying Tn5 and flanking sequences from LS156, in pBluescript II SK, Km^r Ap^r	This study
pCEA1	1766-bp *Eco*RI-*Cla*I fragment from pBEA1 in pBluescript II SK, Ap^r	This study
pPEA1	697-bp *Eco*RI-*Pst*I fragment from pBEA1 in pBluescript II SK, Ap^r	This study
pGTA1	2.1-kb fragment, PCR amplified from LS155 in pGEM-T, Ap^r	This study
pLXABA	834-bp *Eco*RI-*Bam*HI fragment (xabA ORF) from pGTA1 in pLAFR3, Tc^r	This study
pLENTD	630-bp *EcoRI-Hin*dIII fragment (entD ORF) from DH5□ in pLAFR3, Tc^r	This study

TABLE 4

Similarity between XabA and other PPTases involved in antibiotic and fatty acid biosynthesis in bacteria

Pathway	Protein	Organism	Specificity (A/P)†	Domain I Domain II	Homology (ID/SIM)
Albicidin	XabA	X.albilineans	?	GVGIDLERP--(X)39--FSAKESLFKAJ Y	-
Enterobactin	EntD	E.coli	P‡	PIGIDIEEI--(X)36--FSAKESAFKASE	35.9/56.5
		S.flexneri	?	PIGVDIEEI--(X)36--FSAKESAFKAS?	35.0/55.6
		S.typhimurium	?	RIGIDIEKI--(X)35--FSAKESVYKAFQ	35.9/62.0
		S.austin	?	RVGVDIEKI--(X)35--FSAKESVYKA?Q	36.1/61.1
Mycobactin	PptT	M.tuberculosis	P	SVGIDAEPH--(X)34--FCAKEATYKAWF	30.5/55.5
Surfactin	Sfp	B.subtilis	A/P‡	PIGIDIEKT--(X)35--WSMKESFIKQE3	24.8/48.5
	Psf-1	B.pumilus	?	PVGIDIEEI--(X)35--WSMKEAFIKLTG	19.8/47.6
Gramicidin	Gsp	B.brevis	P‡	PVGIDIERI--(X)35--WTIKESYIKAIG	20.8/42.0
Iturin A	Lpa-14	B.subtilis	?	PIGIDIEKM--(X)35--WSMKESFIKQAG	20.0/43.4
Fatty acids	HI0152	H. influenzae	?	AVGIDIEFP--(X)34--WCLREAVLKSQG	19.7/45.7
	AcpS	E. coli	A‡	GLGTDIVEI--(X)40--FAVKEAAAKAFG	16.5/38.8
		M.tuberculosis	A	GVGIDLVSI--(X)41--WAAKEAVIKAWS	25.7/47.6
		B. subtilis	?	GIGLDITEL--(X)41--FAAKEAFSKAFG	25.5/46.2
PPTase domain*				(V/I)G(I/V)D (F/W)(S/C/T)XKE(S/A)XXK	

TABLE 5

Bacterial strains, and plasmids for Example 3

Strain or plasmids	*Characteristics*	*Reference or source*
Strain		
E. coli		
DH5α	Φ80dlacZΔM15, Δ(lacZYA-argF)U169	Promega
JM109	[F', lacI^{q}ZΔM15], Δ(lac-proAB)	Promega
TOP10	F', Δ(mrr-hsdRMS-mcrBC), Δ(are-leu)7697, ΔlacX74	Invitrogen
S17-1λpir	S17-1 lysogenized with λpir	Penfold and Pemberton (1992)
X. albilineans		
Xa13	wt albicidin producer from sugarcane (Queensland), Ap^r	Our laboratory
LS155	wt albicidin producer from sugarcane (Queensland), Ap^r	Wall and Birch (1997)
LS157	xabB::Tn5, albicidin deficient (Tox$^-$), Km^r Ap^r	Wall and Birch (1997)
LS-JP1	thp::pJP-BAS, albicidin deficient (Tox$^-$), Km^{r}Ap^r	This work
LS-JP2	xabC::pJP-BEC, albicidin deficient (Tox$^-$), Km^{r}Ap^r	This work
Plasmids		
pBluescript II SK	ColE1 origin, E. coli cloning vector, Ap^r	Stratagene
pZErO-2	ColE1 origin, E. coli cloning vector, Km^r	Invitrogene
pRK2013	ColE1origin, IncP, Tra$^+$, helper plasmid, Km^r	Ditta *et al.* (1980)
pLAFR3	RK2 origin, Tra$^-$, Mob$^+$, broad host-range cosmid, Tc^r	Staskawicz *et al.* (1987)
pJP5603	Bacterial suicide vector, Km^r	Penfold and Pemberton (1991)
pZIXC	1 kb IPCR product in pZErO-2, Km^r	This work
pBAS	278 bp ApaI-SalI fragment of thp in pBluescrpt II SK, Ap^r	This work

Strain or plasmids	*Characteristics*	*Reference or source*
pJP-BAS	284 bp SalI-KpnI fragment from pBAS in pJP5606, Km^r	This work
pBEC	625 bp ClaI-EcoRI fragment of xabC in pBluescript II SK, Ap^r	This work
pJP-BEC	655 bp EcoRI-KpnI fragment from pBEC in pJP5603, Km^r	This work
pLTHP	1226 bp EcoRI-BamHI fragment from pLXABB in pLAFR3, Tc^r	This work
pLXABC	1029 bp xabC ORF amplified from LS155 in pLAFR3, Tc^r	This work
pLXABB	16.5 kb EcoRI fragment from Xa13 in pLAFR3, Tc^r	This work

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CLAIMS

1. An isolated polypeptide comprising at least one domain selected from the group consisting of:

(a) an acyl-CoA ligase (AL) domain comprising a sequence set forth in any one or more of SEQ ID NO: 6 and 8, or variants thereof.

(b) a β-ketoacyl synthase (KS) domain comprising a sequence set forth in any one or more of SEQ ID NO: 10, 12, 14, 16, 18 and 20, or variants thereof;

(c) a β-ketoacyl reductase (KR) domain comprising the sequence set forth SEQ ID NO: 22, or variants thereof;

(d) an acyl carrier protein (ACP) domain comprising a sequence set forth in any one or more of SEQ ID NO: 24, 26 and 28, or variants thereof;

(e) an adenylation (A) domain comprising a sequence set forth in any one or more of SEQ ID NO: 30, 32, 34, 36, 38, 40, 42, 44, 46 and 48, or variants thereof.

(f) a peptidyl carrier protein (PCP) domain comprising a sequence set forth in any one or more of SEQ ID NO: 50 and 52, and variants thereof; and

(g) a condensation (C) domain comprising a sequence set forth in any one or more of SEQ ID NO: 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78 and 80, or variants thereof.

2. The polypeptide of claim 1, wherein the AL domain comprises each of the sequences set forth in SEQ ID NO: 6 and 8, or variants thereof.

3. The polypeptide of claim 1, wherein the KS domain comprises each of the sequences set forth in SEQ ID NO: 10, 12 and 14, or variants thereof.

4. The polypeptide of claim 1, wherein the KS domain comprises each of the sequences set forth in SEQ ID NO: 16, 18 and 20, or variants thereof.

5. The polypeptide of claim 1, wherein the A domain comprises each of the sequences set forth in SEQ ID NO: 30, 32, 34, 36, 38, 40, 42, 44, 46 and 48, or variants thereof.

6. The polypeptide of claim 1, wherein the C domain comprises each of the sequences set forth in SEQ ID NO: 54, 56, 58, 60, 62, 64 and 66, or variants thereof.

7. The polypeptide of claim 1, wherein the C domain comprises each of the sequences set forth in SEQ ID NO: 68, 70, 72, 74, 76, 78 and 80, or variants thereof.

8. The polypeptide of claim 1, wherein the domains are arranged in an N- to C-terminal direction as follows: AL-ACP-KS-KR-ACP-ACP-KS-PCP-C-A-PCP-C.

9. The polypeptide of claim 1, comprising the sequence set forth in SEQ ID NO: 2, or biologically active fragment thereof, or variant or derivative of these.

10. The polypeptide of claim 9, wherein the variant has at least 60% sequence identity to the sequence set forth in SEQ ID NO: 2.

11. The polypeptide of claim 9, wherein the biologically active fragment is at least 6 amino acids in length.

12. An isolated polypeptide comprising at least a biologically active fragment of the sequence set forth in SEQ ID NO: 2 or variant or derivative thereof.

13. The polypeptide of claim 12, wherein the biologically active fragment is at least 6 amino acids in length.

14. The polypeptide of claim 12, wherein the biologically active fragment comprises at least one domain selected from the group consisting of:

(a) an acyl-CoA ligase (AL) domain comprising a sequence set forth in any one or more of SEQ ID NO: 6 and 8, or variants thereof.

(b) a β-ketoacyl synthase (KS) domain comprising a sequence set forth in any one or more of SEQ ID NO: 10, 12, 14, 16, 18 and 20, or variants thereof;

(c) a β-ketoacyl reductase (KR) domain comprising the sequence set forth SEQ ID NO: 22, or variants thereof;

(d) an acyl carrier protein (ACP) domain comprising a sequence set forth in any one or more of SEQ ID NO: 24, 26 and 28, or variants thereof;

(e) an adenylation (A) domain comprising a sequence set forth in any one or more of SEQ ID NO: 30, 32, 34, 36, 38, 40, 42, 44, 46 and 48, or variants thereof.

(f) a peptidyl carrier protein (PCP) domain comprising a sequence set forth in any one or more of SEQ ID NO: 50 and 52, and variants thereof; and

(g) a condensation (C) domain comprising a sequence set forth in any one or more of SEQ ID NO: 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78 and 80, or variants thereof.

15. The polypeptide of claim 13, wherein the AL domain comprises each of the sequences set forth in SEQ ID NO: 6 and 8, or variants thereof.

16. The polypeptide of claim 13, wherein the KS domain comprises each of the sequences set forth in SEQ ID NO: 10, 12 and 14, or variants thereof.

17. The polypeptide of claim 13, wherein the KS domain comprises each of the sequences set forth in SEQ ID NO: 16, 18 and 20, or variants thereof.

18. The polypeptide of claim 13, wherein the A domain comprises each of the sequences set forth in SEQ ID NO: 30, 32, 34, 36, 38, 40, 42, 44, 46 and 48, or variants thereof.

19. The polypeptide of claim 13, wherein the C domain comprises each of the sequences set forth in SEQ ID NO: 54, 56, 58, 60, 62, 64 and 66, or variants thereof.

20. The polypeptide of claim 13, wherein the C domain comprises each of the sequences set forth in SEQ ID NO: 68, 70, 72, 74, 76, 78 and 80, or variants thereof.

21. The polypeptide of claim 12, wherein the variant has at least 60% sequence identity to said at least a biologically active fragment.

22. The polypeptide of claim 12, wherein the variant has at least 70% sequence identity to any one of the amino acid sequences set forth in SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78 or 80.

23. An isolated polypeptide comprising at least biologically active fragment of the sequence set forth in SEQ ID NO: 83, or a variant or derivative thereof.

24. The polypeptide of claim 23, wherein the biologically active fragment comprises at least one of the consensus PPTase sequence motifs set forth in SEQ ID NO: 89 or 93, or variant thereof.

25. The polypeptide of claim 24, wherein the biologically active fragment comprises both the consensus PPTase sequence motifs set forth in SEQ ID NO: 89 or 93, or variant thereof.

26. The polypeptide of claim 23, wherein the biologically active fragment comprises the intervening sequence between said consensus PPTase sequence motifs, which intervening sequence comprises the sequence set forth in SEQ ID NO: 91, or variant thereof.

27. The polypeptide of claim 23, wherein the biologically active fragment comprises a contiguous sequence of amino acids contained within the sequence set forth in SEQ ID NO: 87, or variant thereof.

28. The polypeptide of claim 23, wherein the biologically active fragment is at least 6 amino acids in length.

29. The polypeptide of claim 23, wherein the variant has at least 60% sequence identity to the sequence set forth in SEQ ID NO: 83.

30. The polypeptide of claim 23, wherein the variant has at least 70% sequence identity to any one of the amino acid sequences set forth in SEQ ID NO: 87, 89, 91 or 93.

31. An isolated polypeptide comprising at least biologically active fragment of the sequence set forth in SEQ ID NO: 95, or a variant or derivative thereof.

32. The polypeptide of claim 31, wherein the biologically active fragment comprises at least one of the consensus methyltransferase sequence motifs set forth in SEQ ID NO: 99, 101 or 103, or variant thereof.

33. The polypeptide of claim 31, wherein the biologically active fragment comprises all the consensus methyltransferase sequence motifs set forth in SEQ ID NO: 99, 101 and 103, or variant thereof.

34. The polypeptide of claim 31, wherein the biologically active fragment comprises a contiguous sequence of amino acids contained within the sequence set forth in SEQ ID NO: 105, or variant thereof.

35. The polypeptide of claim 31, wherein the biologically active fragment comprises a contiguous sequence of amino acids contained within the sequence set forth in SEQ ID NO: 107, or variant thereof.

36. The polypeptide of claim 31, wherein the biologically active fragment is at least 6 amino acids in length.

37. The polypeptide of claim 31, wherein the variant has at least 60% sequence identity to the sequence set forth in SEQ ID NO: 95.

38. The polypeptide of claim 31, wherein the variant has at least 70% sequence identity to any one of the amino acid sequences set forth in SEQ ID NO: 99, 101 or 103.

39. An isolated polynucleotide comprising a sequence encoding at least one domain selected from the group consisting of:

(a) an acyl-CoA ligase (AL) domain comprising a sequence set forth in any one or more of SEQ ID NO: 6 and 8, or variants thereof.

(b) a β-ketoacyl synthase (KS) domain comprising a sequence set forth in any one or more of SEQ ID NO: 10, 12, 14, 16, 18 and 20, or variants thereof;

(c) a β-ketoacyl reductase (KR) domain comprising the sequence set forth SEQ ID NO: 22, or variants thereof;

(d) an acyl carrier protein (ACP) domain comprising a sequence set forth in any one or more of SEQ ID NO: 24, 26 and 28, or variants thereof;

(e) an adenylation (A) domain comprising a sequence set forth in any one or more of SEQ ID NO: 30, 32, 34, 36, 38, 40, 42, 44, 46 and 48, or variants thereof.

(f) a peptidyl carrier protein (PCP) domain comprising a sequence set forth in any one or more of SEQ ID NO: 50 and 52, and variants thereof; and

(g) a condensation (C) domain comprising a sequence set forth in any one or more of SEQ ID NO: 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78 and 80, or variants thereof.

40. The polynucleotide of claim 39, wherein the AL domain is encoded by a nucleotide sequence set forth in any one or more of SEQ ID NO: 5 or 7, or variants thereof.

41. The polynucleotide of claim 40, wherein the AL domain is encoded by a nucleotide sequence comprising each of the sequences set forth in SEQ ID NO: 5 and 7, or variants thereof.

42. The polynucleotide of claim 39, wherein the KS domain is encoded by a nucleotide sequence set forth in any one or more of SEQ ID NO: 9, 11, 13, 15, 17 and 19, or variants thereof.

43. The polynucleotide of claim 42, wherein the KS domain is encoded by a nucleotide sequence comprising each of the sequences set forth in SEQ ID NO: 9, 11 and 13, or variants thereof.

44. The polynucleotide of claim 42, wherein the KS domain is encoded by a nucleotide sequence comprising each of the sequences set forth in SEQ ID NO: 15, 17 and 19, or variants thereof.

45. The polynucleotide of claim 39, wherein the KR domain is encoded by a nucleotide sequence set forth in SEQ ID NO: 21, or variant thereof.

46. The polynucleotide of claim 39, wherein the ACP domain is encoded by a nucleotide sequence set forth in any one or more of SEQ ID NO: 23, 25 and 27, or variants thereof.

47. The polynucleotide of claim 39, wherein the A domain is encoded by a nucleotide sequence set forth in any one or more of SEQ ID NO: 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47, or variants thereof.

48. The polynucleotide of claim 47, wherein the A domain is encoded by a nucleotide sequence comprising each of the sequences set forth in SEQ ID NO: 29, 31, 33, 35, 37, 39, 41, 43, 45 and 47, or variants thereof.

49. The polynucleotide of claim 39, wherein the PCP domain is encoded by a nucleotide sequence set forth in any one or more of SEQ ID NO: 49 and 51, or variants thereof.

50. The polynucleotide of claim 39, wherein the C domain is encoded by a nucleotide sequence set forth in any one or more of SEQ ID NO: 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77 and 79, or variants thereof.

51. The polynucleotide of claim 50, wherein the C domain is encoded by a nucleotide sequence comprising each of the sequences set forth in SEQ ID NO: 53, 55, 57, 59, 61, 63 and 65, or variants thereof.

52. The polynucleotide of claim 50, wherein the C domain is encoded by a nucleotide sequence comprising each of the sequences set forth in SEQ ID NO: 67, 69, 71, 73, 75, 77 and 79, or variants thereof.

53. The polynucleotide of claim 39, comprising the sequence set forth in any one of SEQ ID NO: 1 or 3, or a biologically active fragment thereof at least 18 nucleotides in length, or a polynucleotide variant of these.

54. The polynucleotide of claim 53, wherein the polynucleotide variant has at least 60% sequence identity to any one of the polynucleotides set forth in SEQ ID NO: 1 or 3.

55. The polynucleotide of claim 53, wherein the polynucleotide variant is capable of hybridising to any one of the polynucleotides identified by SEQ ID NO: 1 or 3 under at least low stringency conditions.

56. The polynucleotide of claim 39, wherein the polynucleotide variant comprises a nucleotide sequence encoding at least one said domain.

57. The polynucleotide of claim 56, wherein the nucleotide sequence variant has at least 60% sequence identity to any one or more of the sequences set forth in SEQ ID NO: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77 and 79.

58. The polynucleotide of claim 56, wherein the nucleotide sequence variant is capable of hybridising to any one of the sequences identified by SEQ ID NO: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77 and 79 under at least low stringency conditions.

59. An isolated polynucleotide comprising a sequence encoding at least biologically active fragment of the sequence set forth in SEQ ID NO: 83, or a variant or derivative thereof.

60. The polynucleotide of claim 59, comprising the sequence set forth in any one of SEQ ID NO: 82 and 84, or a biologically active fragment thereof, or a polynucleotide variant of these.

61. The polynucleotide of claim 59, comprising a contiguous sequence of nucleotides at least 18 nucleotides in length and contained within the sequence set forth in SEQ ID NO: 86, or variant thereof.

62. The polynucleotide of claim 59, wherein the polynucleotide variant has at least 60% sequence identity to any one of the polynucleotides set forth in SEQ ID NO: 82, 84 and 86.

63. The polynucleotide of claim 59, wherein the polynucleotide variant is capable of hybridising to any one of the polynucleotides identified by SEQ ID NO: 82, 84 and 86 under at least low stringency conditions.

64. The polynucleotide of claim 59, wherein the polynucleotide variant comprises a nucleotide sequence encoding at least one PPTase sequence motif selected from SEQ ID NO: 89 and 93, or variant thereof.

65. The polynucleotide of claim 64, wherein the polynucleotide variant comprises a nucleotide sequence encoding the intervening sequence between the said consensus PPTase sequence motifs, said nucleotide sequence comprising the sequence set forth in SEQ ID NO: 91.

66. The polynucleotide of claim 59, wherein the polynucleotide variant suitably comprises a nucleotide sequence encoding a contiguous sequence of amino acids contained within the sequence set forth in SEQ ID NO: 87, or variant thereof.

67. The polynucleotide of claim 66, wherein the contiguous sequence is encoded by the sequence set forth in SEQ ID NO: 86, or nucleotide sequence variant thereof displaying at 60% identity thereto.

68. The polynucleotide of claim 64, wherein the PPTase sequence motif is encoded by a nucleotide sequence comprising the sequence set forth in any one of SEQ ID NO: 88 and 92, or nucleotide sequence variant thereof displaying at 60% identity thereto.

69. The polynucleotide of claim 65, wherein the intervening sequence is encoded by the nucleotide sequence set forth in SEQ ID NO: 90, or nucleotide sequence variant thereof displaying at 60% identity thereto.

70. The polynucleotide of claim 66, wherein the contiguous sequence is encoded by the sequence set forth in SEQ ID NO: 86, or nucleotide sequence variant thereof displaying at 60% capable of hybridising thereto under at least low stringency conditions.

71. The polynucleotide of claim 64, wherein the PPTase sequence motif is encoded by a nucleotide sequence comprising the sequence set forth in any one of SEQ ID NO: 88 and 92, or nucleotide sequence variant thereof capable of hybridising thereto under at least low stringency conditions.

72. The polynucleotide of claim 65, wherein the intervening sequence is encoded by the nucleotide sequence set forth in SEQ ID NO: 90, or nucleotide sequence variant thereof capable of hybridising thereto under at least low stringency conditions.

73. An isolated polynucleotide comprising a sequence encoding at least biologically active fragment of the sequence set forth in SEQ ID NO: 95, or a variant or derivative thereof.

74. The polynucleotide of claim 73, comprising the sequence set forth in any one of SEQ ID NO: 94 and 96, or a biologically active fragment thereof, or a polynucleotide variant of these.

75. The polynucleotide of claim 73, comprising a contiguous sequence of nucleotides contained within the sequence set forth in SEQ ID NO: 104, or variant thereof.

76. The polynucleotide of claim 73, comprising a contiguous sequence of nucleotides contained within the sequence set forth in SEQ ID NO: 106, or variant thereof.

77. The polynucleotide of claim 73, wherein the polynucleotide variant has at least 60% sequence identity to any one of the polynucleotides set forth in SEQ ID NO: 94, 96, 104 and 106.

78. The polynucleotide of claim 73, wherein the polynucleotide variant is capable of hybridising to any one of the polynucleotides identified by SEQ ID NO: 94, 96, 104 and 106 under at least low stringency conditions.

79. The polynucleotide of claim 73, wherein the polynucleotide variant comprises a nucleotide sequence encoding a methyltransferase sequence motif selected from any one or more of SEQ ID NO: 99, 101 and 103, or variant thereof.

80. The polynucleotide of claim 79, wherein the methyltransferase sequence motif is encoded by a nucleotide sequence comprising the sequence set forth in any one of SEQ ID NO: 98, 100 and 102, or nucleotide sequence variant thereof displaying at least 60% identity thereto.

81. The polynucleotide of claim 79, wherein the methyltransferase sequence motif is encoded by a nucleotide sequence comprising the sequence set forth in any one of SEQ ID NO: 98, 100 and 102, or nucleotide sequence variant thereof capable of hybridising thereto under at least low stringency conditions.

82. An expression vector comprising the polynucleotide of any one of claims 39, 59 or 73, wherein the polynucleotide is operably linked to a regulatory polynucleotide.

83. A host cell containing the expression vector of claim 82.

84. A multiplicity of cell colonies, constituting a library of colonies, wherein each colony of the library contains an expression vector for the production of the polypeptide of claim 1 or claim 12.

85. A method for enhancing the level and/or functional activity of an albicidin, said method comprising:

- introducing into an albicidin-producing host cell (1) an agent that modulates the expression of a gene encoding at least a portion of the polypeptide of claim 1 or variant or derivative thereof, or the level and/or functional activity of an expression product of said gene, or (2) a vector from which a polynucleotide encoding at least a portion of the polypeptide of claim 1 or variant or derivative thereof can be translated;
- and culturing the host cell for a time and under conditions sufficient to enhance the level and/or functional activity of said albicidin.

86. The method of claim 85, further comprising introducing into said host cell a vector from which a PPTase can be translated.

87. The method of claim 86, wherein the PPTase is selected from EntD or XabA.

88. The method of claim 85, further comprising introducing into said host cell a vector from which a methyltransferase can be translated.

89. The method of claim 86, wherein the methyltransferase is XabC.

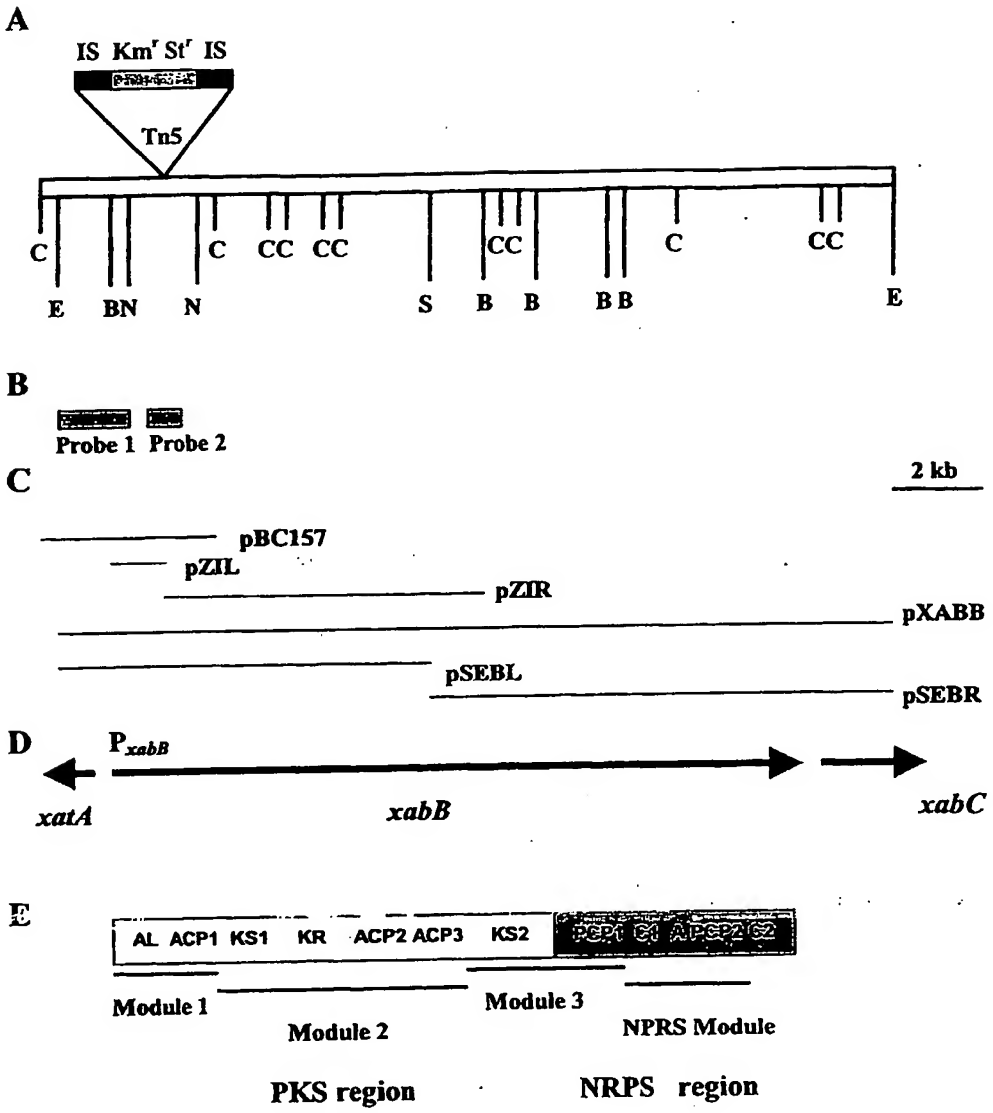
90. An antigen-binding molecule that is immuno-interactive with the polypeptide of claim 1 or claim 12.

91. An antigen-binding molecule that is immuno-interactive with the polypeptide of claim 23.

92. An antigen-binding molecule that is immuno-interactive with the polypeptide of claim 31.

93. A method of preparing a polynucleotide encoding a modified PKS, comprising using a nucleotide sequence encoding the polypeptide of claim 1 or claim 12 as a scaffold and modifying the portions of the nucleotide sequence that encode enzymatic activities, either by mutagenesis, inactivation, deletion, insertion, or replacement.

94. A method for producing polyketides, comprising expressing the modified albicidin PKS encoding nucleotide sequence produced by the method of claim 93 in a suitable host cell to thereby produce a polyketide different from that produced by said polypeptide.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2 kb
pBC157
pZIL
pZIR
pXABB
pSEBL
pSEBR
D
P xabB
xatA
xabB
xabC
E
AL ACP1 KS1 KR ACP2 ACP3 KS2
PCP1 C1 APCP2 C2
Module 1
Module 2
Module 3
NPRS Module
PKS region
NRPS region

FIGURE 1

P1F1

967 GGCGATGCGC GCACACTGCA GGTCCATCAC GCCACCTCCA GCAGGGTGTC
CCGCTACGCG CGTGTGACGT CCAGGTAGTG CGGTGGAGGT CGTCCCACAG
A I R A C Q L D M M RBS
xatA ◄

1017 ATACACGGCC AGCGGATGCT GCAGGTTTTC CACTGGCAGG GCCACTGGCT
TATGTGCCGG TCGCCTACGA CGTCCAAAAG GTGACCGTCC CGGTGACCGA

-35 (P_{xabB}) -10 (P_{xabB})
1067 GTCGTAAGGG AAGCGGTGCC TTGAGCGCCG GTGCGGACAG TATAACGACA
CAGCATTCCC TTCGCCACGG AACTCGCGGC CACGCCTGTC ATATTGCTGT
-10 (P_{xatA})

1117 CGTTCCTTGG CCAAGCGCAC TGTCGGCACG GCCTTGCTGA TGCCGCCCAT
GCAAGGAACC GGTTCGCGTG ACAGCCGTGC CGGAACGACT ACGGCGGGTA
-35 (P_{xatA})

1167 GTAGCCGCGC GCCTGGATCT CGCGTAGTAG CACCACGCTG GCCGGGATCC
CATCGGCGCG CGGACCTAGA GCGCATCATC GTGGTGCGAC CGGCCCTAGG
P1R

RBS *xabB* ►
1217 ATCGAGGGCG CGCTTGCCCA ATGCGCTCAT GCAGATAACT CTTGTAGCCG
TAGCTCCCGC GCGAACGGGT TACGCGAGTA CGTCTATTGA GAACTACGGC
M P N A L M Q I T L V A

FIGURE 2

(i). AL

TSGSSGESKGILLSH--GYFRTGDL Xal-XabB(AL)
TGGTTGVAKGAMLTH--GWMATGDI Hin-LCFA
TSGSTGTPKAVMLNH--GWFETGDL Bsu-PksJ
SSGSTGDPKGVMLTH--GWVKTGDL Bsu-MycA(AL)
SSGTTGLPKGVMLTH--GWLHTGDI Pcr-ComL2
TSGTTGRPKGVVSAQ--GWYRTGDL Sma-FkbB(AL)
TSGTTGRPKGVVSTQ--GWFRTGDL Ame-RifA(AL)
TSGTTGTPKGVLSTQ--GWYRTGDL Shy-RapA(AL)

(ii). KS

GPSEVINSACSSSLVAL--VELHGTGTSL--ALGHLGAAAG Xal-XabB (KS1)
GPSLAVDTACSASLTAI--IEAHGTGTVL--NIGHAESAAG Xal-XabB (KS2)
GPSLFVHTNCSSSLSAL--VEAHGTGTLL--NLGHLDTVAG Mxa-Ta1
GPAVTVDTACSSSLVAV--IEAHGTGTKL--NIGHLFEAAG Bsu-MycA
GPAVTVDTACSSSLVAL--VEAHGTGTRL--NIGHAQAAAG Ser-EryA1
GPAMTVDTACSSGLTAL--VEAHGTGTRL--NIGHTQAAAG Ser-EryA3
GPSVLVDTACSGGLTAL--VECHGTGTQA--NIGHLEGASG Che-PKS1
GPSLAVDTACSSSLTAI--LEAHGTGTAL--NIGHCESAAG Bsu-PksM
GPSVAVDTACSSSLVAI--VEAHGTGTLL--NLGHTEAAAG Mtu-PpsA
GPSLTIDTACSSSLMAL--VEAHGTGTKV--NMGHPEPASG Chick-FAS
GPSIALDTACSSSLLAL--IEAHGTGTKV--NMGHPEPASG Rat-FAS
* * *
(Active site cysteine) (Active site histidine)

(iii). KR

VYVVIGGAGGLGEVLSEHLIRTYD.AQLIWIGR Xal-XabB
VYVISGGTGALARLFVAEIGKRATRATVILVAR Mxa-Ta1
TVLVTGGTGGVGGQIARWLARRG.APHLLLVSR Ser-EryA1
TVLVTGGTGGIGAHLARWLARSG.AEHLVLLGR Ser-EryA3
SYLLVGGVGGLGSATALAMSTRG.ARHLLLINR Che-PKS1
SYIITGGLGGLGLFFASKLAAAG.CGRIVLTAR Mtu-MAS
SYIITGGLGGFGLELAQWLIERG.AQKLVLTSR Chick-FAS
SYIITGGLGGFGLELARWLVLRG.AQRLVLTSR Rat-FAS

(iv). ACP

CELALDSLQCVR Xal-XabB(ACP1)
EYYGVDSIVAIE Xal-XabB (ACP2)
ESYGVDSIVIIE Xal-XabB (ACP3)
IGFGLDSIMLTQ Bsu-MycA
ERYGIDSIIITQ Mxa-Ta1
AELGVDSLSALE Ser-EryA1
QDYGIDSLVAVE Che-PKS1
IEYGLDSLGMLE Mtu-MAS
ADLGLDSLMGVE Chick-FAS
ADLGLDSLMGVE Rat-FAS
* (Active site serine)

FIGURE 3

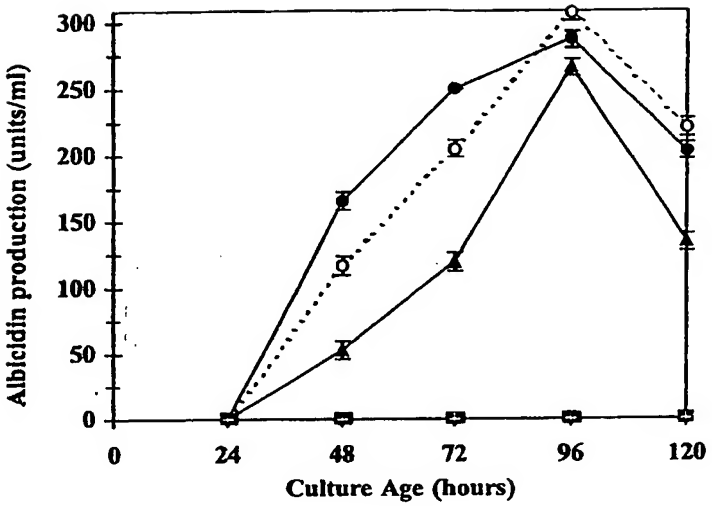
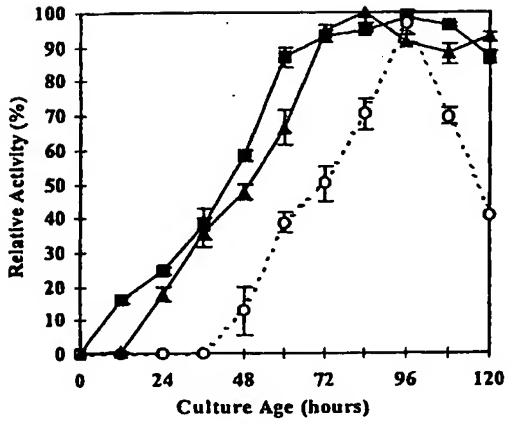

Albicidin production (units/ml)
300
250
200
150
100
50
0
0
24
48
72
96
120
Culture Age (hours)

FIGURE 4

A


Relative Activity (%)
100
90
80
70
60
50
40
30
20
10
0
0
24
48
72
96
120
Culture Age (hours)

B

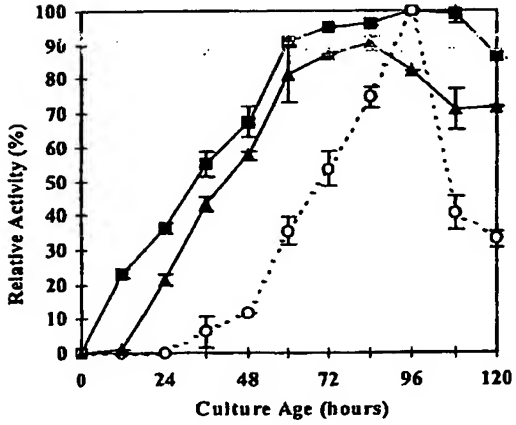
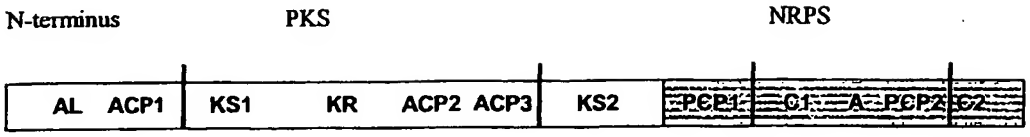

Relative Activity (%)
100
90
80
70
60
50
40
30
20
10
0
0
24
48
72
96
120
Culture Age (hours)

FIGURE 5

A. *X. albilineans* XabB (4801 aa)



B. *B. subtilis* MycA (3971 aa)

C. *Yersinia pestis* HMWP1 (3163 aa)

D. *M. xanthus* Ta1 (2392 aa)

E. *B. subtilis* PksorfX6 (4447 aa)

FIGURE 6

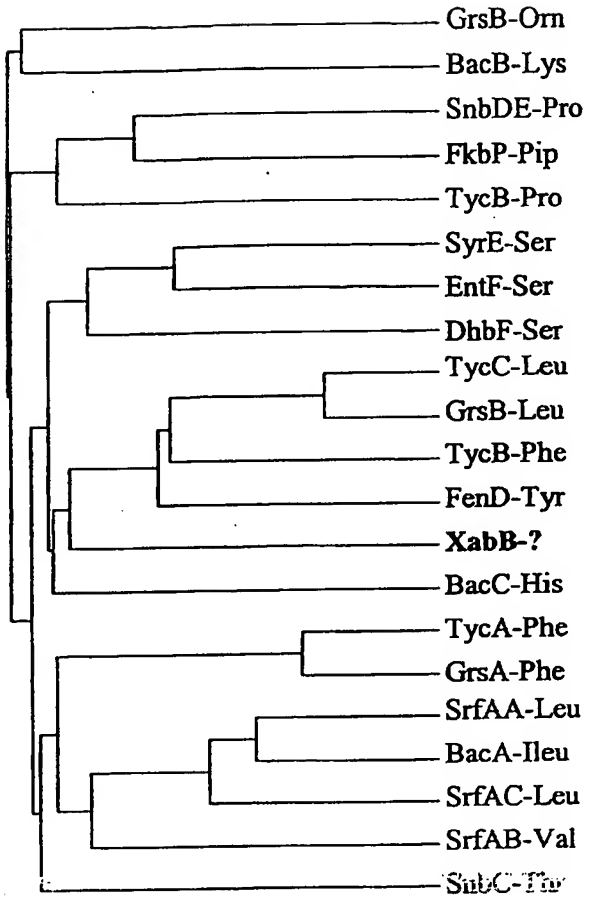

GrsB-Orn
BacB-Lys
SnbDE-Pro
FkbP-Pip
TycB-Pro
SyrE-Ser
EntF-Ser
DhbF-Ser
TycC-Leu
GrsB-Leu
TycB-Phe
FenD-Tyr
XabB-?
BacC-His
TycA-Phe
GrsA-Phe
SrfAA-Leu
BacA-Ileu
SrfAC-Leu
SrfAB-Val

FIGURE 7

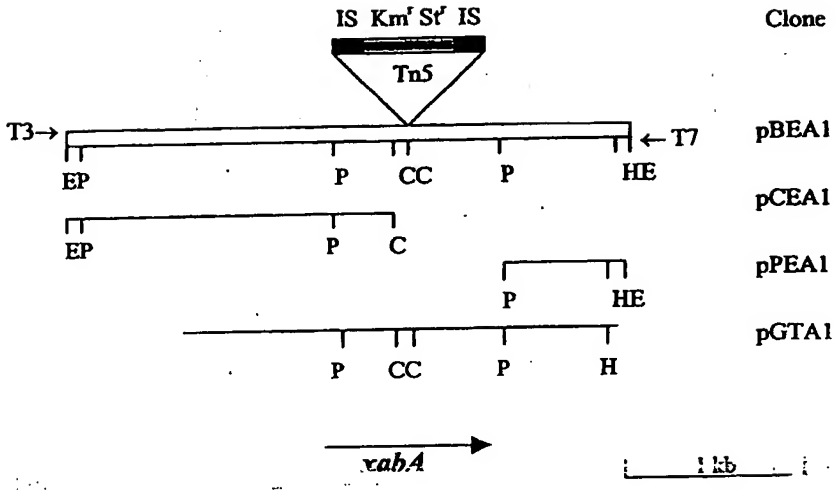
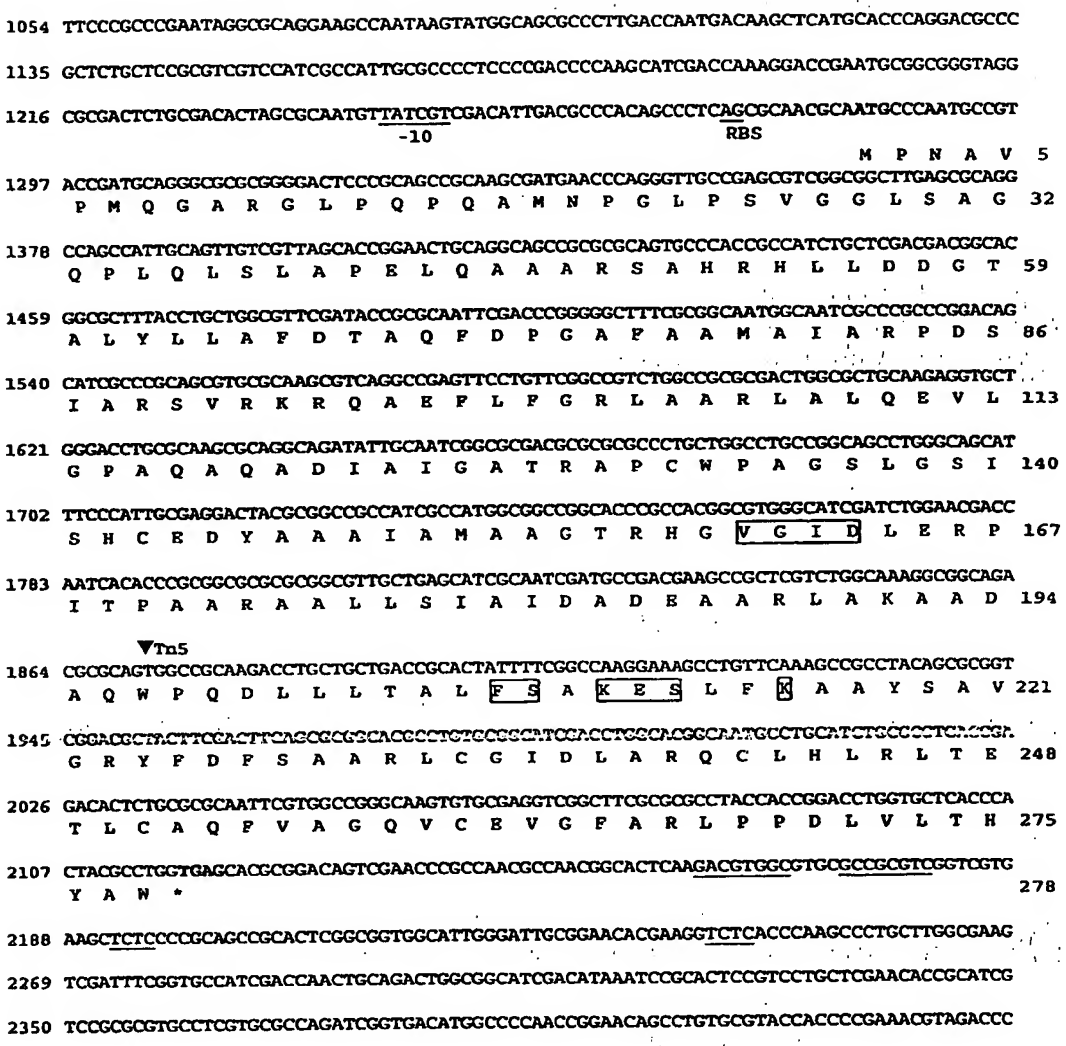

IS Km^r St^r IS
Clone
Tn5
T3→
← T7
pBEA1
EP
P
CC
P
HE
pCEA1
EP
P
C
pPEA1
P
HE
pGTA1
P
CC
P
H
vabA
1 kb

FIGURE 8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1216 CGCGACTCTGCGACACTAGCGCAATGTTATCGTCGACATTGACGCCCACAGCCCTCAGCGCAACGCAATGCCCAATGCCGT
-10
RBS
M P N A V 5
1297 ACCGATGCAGGGCGCGCGGGGACTCCCGCAGCCGCAAGCGATGAACCCAGGGTTGCCGAGCGTCGGCGGCTTGAGCGCAGG
P M Q G A R G L P Q P Q A M N P G L P S V G G L S A G 32
1378 CCAGCCATTGCAGTTGTCGTTAGCACCGGAACTGCAGGCAGCCGCGCGCAGTGCCCACCGCCATCTGCTCGACGACGGCAC
Q P L Q L S L A P E L Q A A A R S A H R H L L D D G T 59
1459 GGCGCTTTACCTGCTGGCGTTCGATACCGCGCAATTCGACCCGGGGGCTTTCGCGGCAATGGCAATCGCCCGCCCGGACAG
A L Y L L A F D T A Q F D P G A F A A M A I A R P D S 86
1540 CATCGCCCGCAGCGTGCGCAAGCGTCAGGCCGAGTTCCTGTTCGGCCGTCTGGCCGCGCGACTGGCGCTGCAAGAGGTGCT
I A R S V R K R Q A E F L F G R L A A R L A L Q E V L 113
1621 GGGACCTGCGCAAGCGCAGGCAGATATTGCAATCGGCGCGACGCGCGCGCCCTGCTGGCCTGCCGGCAGCCTGGGCAGCAT
G P A Q A Q A D I A I G A T R A P C W P A G S L G S I 140
1702 TTCCCATTGCGAGGACTACGCGGCCGCCATCGCCATGGCGGCCGGCACCCGCCACGGCGTGGGCATCGATCTGGAACGACC
S H C E D Y A A A I A M A A G T R H G V G I D L E R P 167
1783 AATCACACCCGCGGCGCGCGCGGCGTTGCTGAGCATCGCAATCGATGCCGACGAAGCCGCTCGTCTGGCAAAGGCGGCAGA
I T P A A R A A L L S I A I D A D E A A R L A K A A D 194
▼Tn5
1864 CGCGCAGTGGCCGCAAGACCTGCTGCTGACCGCACTATTTTCGGCCAAGGAAAGCCTGTTCAAAGCCGCCTACAGCGCGGT
A Q W P Q D L L L T A L F S A K E S L F K A A Y S A V 221
1945 CGGACGCTACTTCGACTTCAGCGCGGCACGCCTGTCCGCGATCGACCTGGCACGGCAATGCCTGCATCTGCCCCTCACCGA
G R Y F D F S A A R L C G I D L A R Q C L H L R L T E 248
2026 GACACTCTGCGCGCAATTCGTGGCCGGGCAAGTGTGCGAGGTCGGCTTCGCGCGCCTACCACCGGACCTGGTGCTCACCCA
T L C A Q F V A G Q V C E V G F A R L P P D L V L T H 275
2107 CTACGCCTGGTGAGCACGCGGACAGTCGAACCCGCCAACGCCAACGGCACTCAAGACGTGGCGTGCGCCGCGTCGGTCGTG
Y A W * 278
2188 AAGCTCTCCCCGCAGCCGCACTCGGCGGTGGCATTGGGATTGCGGAACACGAAGGTCTCACCCAAGCCCTGCTTGGCGAAG
2269 TCGATTTCGGTGCCATCGACCAACTGCAGACTGGCGGCATCGACATAAATCCGCACTCCGTCCTGCTCGAACACCGCATCG
2350 TCCGCGCGTGCCTCGTGCGCCAGATCGGTGACATGGCCCCAACCGGAACAGCCTGTGCGTACCACCCCGAAACGTAGACCC

FIGURE 9

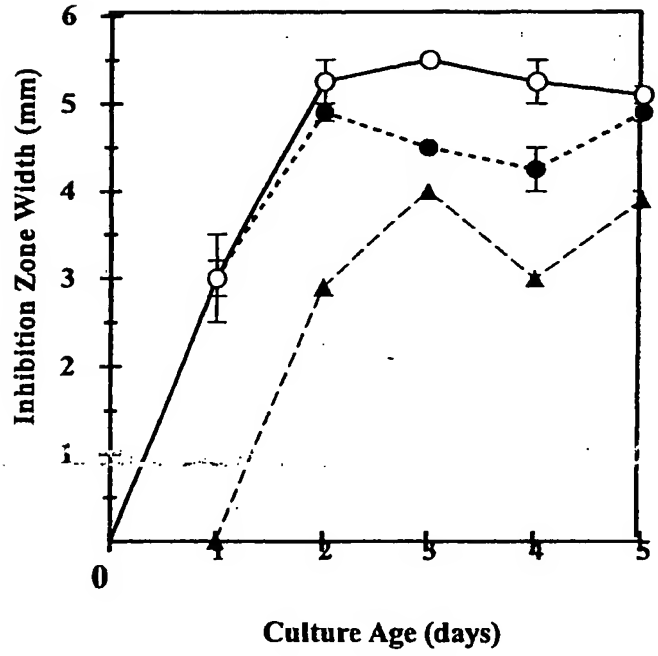


FIGURE 10

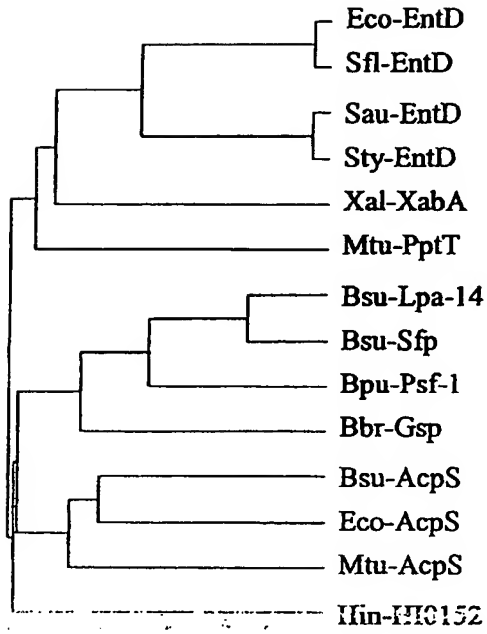

Eco-EntD
Sfl-EntD
Sau-EntD
Sty-EntD
Xal-XabA
Mtu-PptT
Bsu-Lpa-14
Bsu-Sfp
Bpu-Psf-1
Bbr-Gsp
Bsu-AcpS
Eco-AcpS
Mtu-AcpS
Hin-HI0152

FIGURE 11

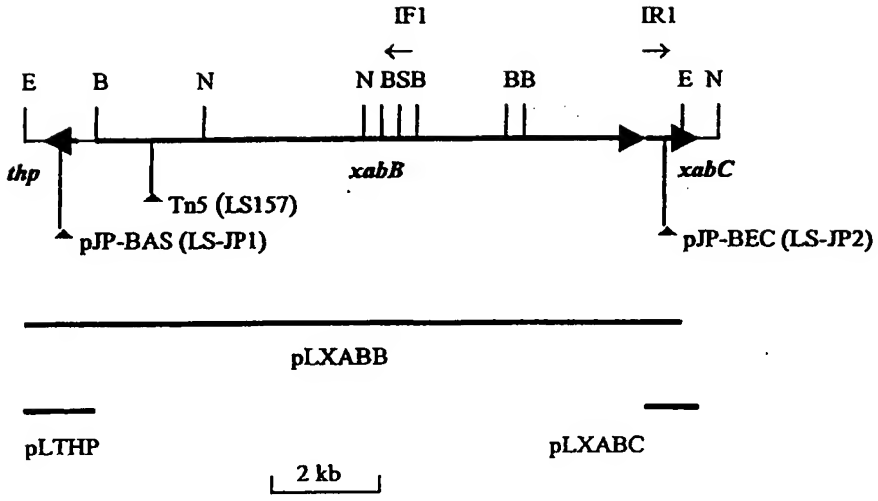
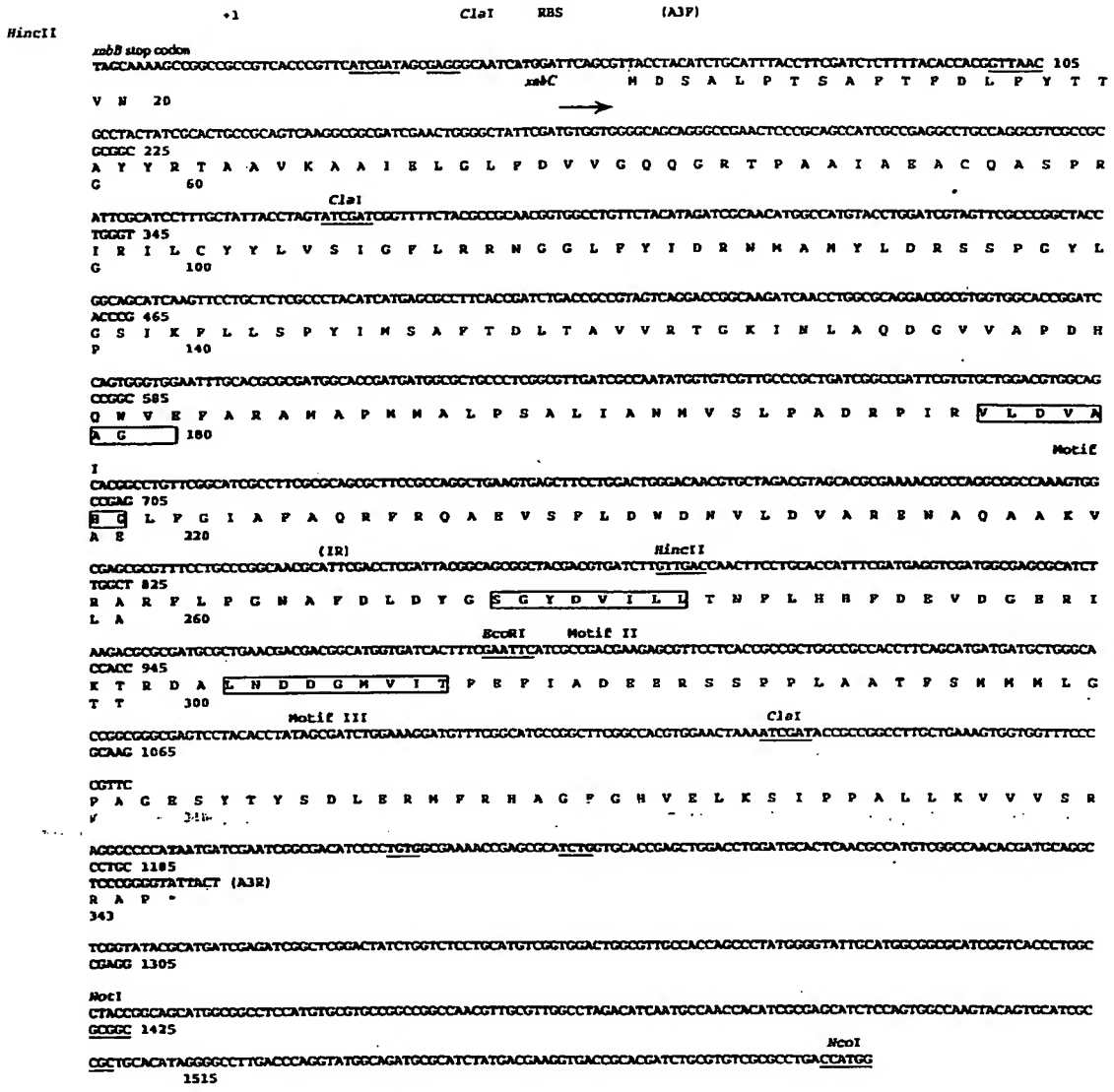

IF1
IR1
E
B
N
N BSB
BB
E N
thp
xabB
xabC
Tn5 (LS157)
pJP-BAS (LS-JP1)
pJP-BEC (LS-JP2)
pLXABB
pLTHP
pLXABC
2 kb

FIGURE 12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ClaI
RBS
(A3P)
HincII
mbB stop codon
mbC
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V N 20
A Y Y R T A A V K A A I E L G L F D V V G Q Q G R T P A A I A E A C Q A S P R
G 60
ClaI
I R I L C Y Y L V S I G F L R R N G G L F Y I D R N M A M Y L D R S S P G Y L
G 100
G S I K F L L S P Y I M S A F T D L T A V V R T G K I N L A Q D G V V A P D H
P 140
Q W V E F A R A M A P M M A L P S A L I A N M V S L P A D R P I R V L D V A
A G 180
Motif I
R G L F G I A F A Q R F R Q A E V S F L D W D N V L D V A R E N A Q A A K V
A E 220
(IR)
HincII
R A R F L P G N A F D L D Y G S G Y D V I L T N F L H H F D E V D G E R I
L A 260
EcoRI
Motif II
K T R D A L N D D G M V I T P E F I A D E E R S S P P L A A T F S M M M L G
T T 300
Motif III
ClaI
P A G E S Y T Y S D L E R M F R H A G F G H V E L K S I P P A L L K V V V S R
V 340
R A P *
343
NotI
NcoI
1515

FIGURE 13

Xal-XabC	174	VLDVAAGHG	236	SGYDVILL	267	ALNDDGMVIT
Sgl-TcmO	173	FVDLGGARG	234	PRADVFIV	263	ALTPGGAVLV
Sgl-TcmN	331	IADLGGGDG	393	TGYDAYLF	423	IGDDDARLLI
Smy-MdmC	64	VLEIGTFTG	135	GAFDIVFV	159	LVRPGGLVAI
Mxa-SafC	63	TLEVGVFTG	134	GTFDLAFI	158	LVRPGGLIIL
Ser-EryG	85	VLDVGFGLG	149	ETFDRVTS	178	VLKPGGVLAI
Spe-DauK	183	VLDVGGGKG	254	RKADAIIL	273	ALEPGGRILI
Sal-DmpM	208	VVDIGGADG	269	GGGDLYVL	298	AMPAHARLLV
Shy-RapM	106	VLEVGCGMG	155	VQGDAEEL	194	ALRRGGALSH
Sav-AveD	71	VLDVGCGSG	124	GSFDAAWA	151	VLRPGGRLAV
		Motif I		Motif II		Motif III

FIGURE 14

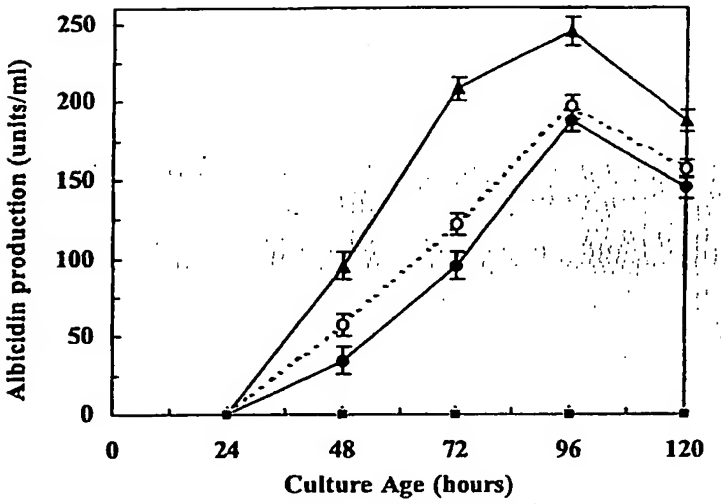
Albicidin production (units/ml)
250
200
150
100
50
0
0
24
48
72
96
120
Culture Age (hours)

FIGURE 15

SEQUENCE LISTING

<110> The University of Queensland (All designated states, except U.S.)
Robert, Birch (U.S. only)

<120> Polynucleotides and polypeptides associated with antibiotic biosynthesis and uses therefor

<130> 2454928/VPA

<140> Not yet assigned
<141> 2001-09-21

<150> AU PR0277/00
<151> 2000-09-21

<150> AU PR0304/00
<151> 2000-09-22

<150> AU PR0320/00
<151> 2000-09-22

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gctttccagc gcccgcatgc ctgccaccca taaagcggtt ctttcgatat ctctcatgca 120

tacgctccgg ttcgtggtcg gcttgcgccg atgcatcata gatatgcatg actcgattcg 180

cggcaccgtg ccttgatggt ggctgcgaag cgaaaacaat aaccaaaggg tggtgctcga 240

cggctttact gtagcgacac cttgtccatc gccttacgga tggtctgatc cacgcaagcg 300

aaagatgaga taaaccacat cagctgtcaa cgccgattta aatttgaccc actttccttt 360

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gaatcgtcga agtaaatctg acccacccgg ggtcttccat cgtcgggctg ctaggctgcg    420

cagggcaaag cccgtcgcag cccagcagcc ctgcgccggc tcacgcccga agggcaggta    480

gccgatctcg tcgaccacca gcagcttcgg ccctagtacc gcgcgattga agtagtcctt    540

cagccggttc tgcgccttga ccgctgccag ttgcatcatc aggtcggccg cggtgatgaa    600

acgtgccttg tgccccgcca tcaccgcacg ctggcacagc gccagggcga tgtgggtctc    660

gccgacaccg ctggggccaa gcatcaccac gttctcggcg cgctcgacga aggtcaggtg    720

gccgagctcg acgatctgcg ccttcgaggc gccgccggcc tgggcccagt cgaactgctc    780

cagcgtcttg atggacggca tcctggcaag tcgcgtcagc accgtgcgct tgcgctcttc    840

acgcgcgagc tgttcgcttg ccagcacctt ctccaggaag tagctggcat cctcgcacgc    900

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ggcacggcct tgctgatgcc gcccatgtag ccgcgcgcct ggatctcgcg tagtagcacc   1200

acgctggccg ggatccatcg agggcgcgc ttg ccc aat gcg ctc atg cag ata      1253
                                 Leu Pro Asn Ala Leu Met Gln Ile
                                 1               5

act ctt gta gcc gtc cag ttt gca ggc gta ttg tta ggc gtc acc gct      1301
Thr Leu Val Ala Val Gln Phe Ala Gly Val Leu Leu Gly Val Thr Ala
    10                  15                  20

cgc gcg gcg atc ccc aat aag gcg ggt atg aga cgc gca tgg ccg ccc      1349
Arg Ala Ala Ile Pro Asn Lys Ala Gly Met Arg Arg Ala Trp Pro Pro
25                  30                  35                  40

ttc ccg cag gcg tgc tgt cgc tct att gct tac ctc atg cag aga tcg      1397
Phe Pro Gln Ala Cys Cys Arg Ser Ile Ala Tyr Leu Met Gln Arg Ser
                45                  50                  55

cca atg tcg ccg tta cag caa acg ctg cta acc cgc ctc gcc agt gcg      1445
Pro Met Ser Pro Leu Gln Gln Thr Leu Leu Thr Arg Leu Ala Ser Ala
            60                  65                  70

gcc gcc tcc cgg aca atg atc gag ttt ccg cgt ccg gag cac gca tcg      1493
Ala Ala Ser Arg Thr Met Ile Glu Phe Pro Arg Pro Glu His Ala Ser
        75                  80                  85

cca caa tgt tgc gac gat gcc gag ctt gcg cga ctg atc gtg cag ttg      1541
Pro Gln Cys Cys Asp Asp Ala Glu Leu Ala Arg Leu Ile Val Gln Leu
    90                  95                  100

tcg gcg gga ctg caa ccg ctg gcg atg ccg ggt acc tac gtg atc att      1589
Ser Ala Gly Leu Gln Pro Leu Ala Met Pro Gly Thr Tyr Val Ile Ile
105                 110                 115                 120

gcc gcg cca cat ggt ggt ttg ttc gcg gca gcc ctg ctt gcc tgt ttg      1637
Ala Ala Pro His Gly Gly Leu Phe Ala Ala Ala Leu Leu Ala Cys Leu
                125                 130                 135
```

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cat gcc aac ctg gtg gcg gtg ccg ttt cca ctg gat gtt gct cag cca      1685
His Ala Asn Leu Val Ala Val Pro Phe Pro Leu Asp Val Ala Gln Pro
            140                 145                 150

aat gag cgg gaa cag gcc agg ctg gag acg atc cac gca caa ttg atg      1733
Asn Glu Arg Glu Gln Ala Arg Leu Glu Thr Ile His Ala Gln Leu Met
        155                 160                 165

gag cat ggc aat gta gcg gtt ctg ctt gac gat gtc gcc gat cgc agt      1781
Glu His Gly Asn Val Ala Val Leu Leu Asp Asp Val Ala Asp Arg Ser
    170                 175                 180

gcc ttc gcg cgc atg gcg cat gct gcg ggc acc ttc ctg gcg acc ttc      1829
Ala Phe Ala Arg Met Ala His Ala Ala Gly Thr Phe Leu Ala Thr Phe
185                 190                 195                 200

gcc gat cta aag cgc gaa tcg acc agc gcc tcc ttg tgc ccg gcg tcg      1877
Ala Asp Leu Lys Arg Glu Ser Thr Ser Ala Ser Leu Cys Pro Ala Ser
                205                 210                 215

cct tcg gac gcc gcc ttg ctg ttg ttt acc tct ggt tcc tcg ggt gag      1925
Pro Ser Asp Ala Ala Leu Leu Leu Phe Thr Ser Gly Ser Ser Gly Glu
            220                 225                 230

tcc aag ggc atc ctg ctt agc cac cgc aac ctg cat cat cag atc cag      1973
Ser Lys Gly Ile Leu Leu Ser His Arg Asn Leu His His Gln Ile Gln
        235                 240                 245

gct ggc atc cgg cag tgg agc ttg gac gag cat agc cat gtg gtg acc      2021
Ala Gly Ile Arg Gln Trp Ser Leu Asp Glu His Ser His Val Val Thr
    250                 255                 260

tgg ctt tct ccc gcg cac aac ttc ggc ctg cat ttc ggc ttg ctg gca      2069
Trp Leu Ser Pro Ala His Asn Phe Gly Leu His Phe Gly Leu Leu Ala
265                 270                 275                 280

ccc tgg ttc agt ggc gcg acg gtc agt ttc atc cat ccg cac agt tat      2117
Pro Trp Phe Ser Gly Ala Thr Val Ser Phe Ile His Pro His Ser Tyr
                285                 290                 295

atg aaa cga ccc ggc ttc tgg ctg gag acg gtt gcg gct aga gac gcc      2165
Met Lys Arg Pro Gly Phe Trp Leu Glu Thr Val Ala Ala Arg Asp Ala
            300                 305                 310

acg cac atg gcc gcg ccg aac ttc gcg ttc gac tac tgc tgc gac tgg      2213
Thr His Met Ala Ala Pro Asn Phe Ala Phe Asp Tyr Cys Cys Asp Trp
        315                 320                 325

gtg atg gtc gag cag ctt ccg ccg tct gcg ttg tct acg ctt acg cat      2261
Val Met Val Glu Gln Leu Pro Pro Ser Ala Leu Ser Thr Leu Thr His
    330                 335                 340

atc gtg tgt ggc ggc gag ccg gtg cgc gcc tcg acc atg cag cgc ttc      2309
Ile Val Cys Gly Gly Glu Pro Val Arg Ala Ser Thr Met Gln Arg Phe
345                 350                 355                 360

ttc gag aaa ttc gcc gga ctc ggt gcg cgt acg cag act ttc atg ccg      2357
Phe Glu Lys Phe Ala Gly Leu Gly Ala Arg Thr Gln Thr Phe Met Pro
                365                 370                 375

cac ttc ggc ttg tct gaa acc ggt gcg ctg agt acc ttg gac gag gcg      2405
His Phe Gly Leu Ser Glu Thr Gly Ala Leu Ser Thr Leu Asp Glu Ala
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                380                 385                 390

ccc caa cag cgc gtc ttg gaa cta gat gcc gac gcc ttg aac aaa cgc      2453
Pro Gln Gln Arg Val Leu Glu Leu Asp Ala Asp Ala Leu Asn Lys Arg
        395                 400                 405

aag cgc gtg gcg gca ggg gcg agc cag gcg cgt gtg aca gtg ctc aat      2501
Lys Arg Val Ala Ala Gly Ala Ser Gln Ala Arg Val Thr Val Leu Asn
    410                 415                 420

tgc ggc gcc gtc gac caa gat gtg gag ttg cgt atc gtc tgt cct gaa      2549
Cys Gly Ala Val Asp Gln Asp Val Glu Leu Arg Ile Val Cys Pro Glu
425                 430                 435                 440

ggc gag acg ttg tgc aga cca gat gag atc ggc gaa ata tgg gta aag      2597
Gly Glu Thr Leu Cys Arg Pro Asp Glu Ile Gly Glu Ile Trp Val Lys
                445                 450                 455

tcg cct gcg atc gcc cgt ggc tac ctg ttt gcg aag ccc gcc gat cag      2645
Ser Pro Ala Ile Ala Arg Gly Tyr Leu Phe Ala Lys Pro Ala Asp Gln
            460                 465                 470

cga cag ttc aac tgc agc atc cgt cat acc gac gat agc ggt tac ttt      2693
Arg Gln Phe Asn Cys Ser Ile Arg His Thr Asp Asp Ser Gly Tyr Phe
        475                 480                 485

cgt acc ggc gac ctg ggt ttc att gcc gat ggc tgt ctg tat gtc acc      2741
Arg Thr Gly Asp Leu Gly Phe Ile Ala Asp Gly Cys Leu Tyr Val Thr
    490                 495                 500

gga agg gta aag gag gtg ctg atc ata cgc ggt aag aat cat tac ccc      2789
Gly Arg Val Lys Glu Val Leu Ile Ile Arg Gly Lys Asn His Tyr Pro
505                 510                 515                 520

gca cat atc gaa gcc tcg atc gcc gct acc gca tcg cct ggc gcg ctg      2837
Ala His Ile Glu Ala Ser Ile Ala Ala Thr Ala Ser Pro Gly Ala Leu
                525                 530                 535

atg ccg gtg gtg ttc agc atc gag cgg cag gac gag gag cgc gta gct      2885
Met Pro Val Val Phe Ser Ile Glu Arg Gln Asp Glu Glu Arg Val Ala
            540                 545                 550

gcg gtg atc gcc gtc aat cac ccg tgg acg ccg gca gca tgc gcc gcg      2933
Ala Val Ile Ala Val Asn His Pro Trp Thr Pro Ala Ala Cys Ala Ala
        555                 560                 565

cag gca cac aag atc cgg caa cag gta gcc gac cag cat gga gtc gcc      2981
Gln Ala His Lys Ile Arg Gln Gln Val Ala Asp Gln His Gly Val Ala
    570                 575                 580

ctg gcg gag cta gcc ttt gcc gaa cac cgg cac gtg ttc ggc acc tat      3029
Leu Ala Glu Leu Ala Phe Ala Glu His Arg His Val Phe Gly Thr Tyr
585                 590                 595                 600

ccg ggc aaa ctg aag cgg cgc cta gtc aag gaa gcc tat gtc aac ggc      3077
Pro Gly Lys Leu Lys Arg Arg Leu Val Lys Glu Ala Tyr Val Asn Gly
                605                 610                 615

cag ctg ccg ttg tta tgg cat gag ggt aag aac cgg gac gta cca gcg      3125
Gln Leu Pro Leu Leu Trp His Glu Gly Lys Asn Arg Asp Val Pro Ala
            620                 625                 630

gcc gcc gcg gac gat cgg cag gcg caa cac gtg gcg gac ctg tgt cgg      3173
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Ala Ala Ala Asp Asp Arg Gln Ala Gln His Val Ala Asp Leu Cys Arg
        635                 640                 645

aag gtc ttt ttg ccg gtg ttg ggt gtc gcg ccg ccg cat gcc caa tgg     3221
Lys Val Phe Leu Pro Val Leu Gly Val Ala Pro Pro His Ala Gln Trp
    650                 655                 660

ccg ctg tgc gaa ctg gcg ctg gat tcg ctc caa tgc gtg cgt ctt gcc     3269
Pro Leu Cys Glu Leu Ala Leu Asp Ser Leu Gln Cys Val Arg Leu Ala
665                 670                 675                 680

ggt gcc atc gaa gag tgc tac ggc gtg cct ttc gaa ccc acg ttg cta     3317
Gly Ala Ile Glu Glu Cys Tyr Gly Val Pro Phe Glu Pro Thr Leu Leu
                685                 690                 695

ttc aag ctt gag acg gtc ggg gca atc gcc gaa tat gtc ctg gcg cac     3365
Phe Lys Leu Glu Thr Val Gly Ala Ile Ala Glu Tyr Val Leu Ala His
            700                 705                 710

gga cgt cag gcg ccc acg ccg acg cgt gcg ccg gtg gca agc aca aca     3413
Gly Arg Gln Ala Pro Thr Pro Thr Arg Ala Pro Val Ala Ser Thr Thr
        715                 720                 725

tgc tca gag gaa ccg atc gcc att gtg gcg atg cac tgt gag gtg ccc     3461
Cys Ser Glu Glu Pro Ile Ala Ile Val Ala Met His Cys Glu Val Pro
    730                 735                 740

gga__cg ggc gag aac act gaa gca ttg tgg tcg ttc ctg cgg agc gac     3509
Gly Ala Gly Glu Asn Thr Glu Ala Leu Trp Ser Phe Leu Arg Ser Asp
745                 750                 755                 760

gtc aac gcg atc cgg ccg atc gaa tca acg cgc ccg gac tta tgg gca     3557
Val Asn Ala Ile Arg Pro Ile Glu Ser Thr Arg Pro Asp Leu Trp Ala
                765                 770                 775

gcg atg cgc gcc tat ccc ggc ctc gcg ggc gaa cag ctg ccg cgc tat     3605
Ala Met Arg Ala Tyr Pro Gly Leu Ala Gly Glu Gln Leu Pro Arg Tyr
            780                 785                 790

gcg ggt ttc ctc gac gac gtt gat gct ttc gat gct gcg ttt ttc ggt     3653
Ala Gly Phe Leu Asp Asp Val Asp Ala Phe Asp Ala Ala Phe Phe Gly
        795                 800                 805

atc tcg cgt cgc gag gcc gaa tgc atg gac ccg cag cag cgc aaa gtg     3701
Ile Ser Arg Arg Glu Ala Glu Cys Met Asp Pro Gln Gln Arg Lys Val
    810                 815                 820

ctg gag atg gtg tgg aag ctg atc gag caa gcc ggt cac gat ccg ctg     3749
Leu Glu Met Val Trp Lys Leu Ile Glu Gln Ala Gly His Asp Pro Leu
825                 830                 835                 840

tcc tgg ggc ggc cag ccg gtc ggc ctg ttc gtg ggt gcg cat acg tcc     3797
Ser Trp Gly Gly Gln Pro Val Gly Leu Phe Val Gly Ala His Thr Ser
                845                 850                 855

gac tat ggc gag ctg ctg gcg agc cag ccg caa ctg atg gcc caa tgt     3845
Asp Tyr Gly Glu Leu Leu Ala Ser Gln Pro Gln Leu Met Ala Gln Cys
            860                 865                 870

ggc gct tac atc gat tcg ggt tcg cat ttg acc atg att ccg aac cgg     3893
Gly Ala Tyr Ile Asp Ser Gly Ser His Leu Thr Met Ile Pro Asn Arg
        875                 880                 885
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gct tcg cgc tgg ttc aat ttc acc ggc ccc agc gaa gta atc aac agc     3941
Ala Ser Arg Trp Phe Asn Phe Thr Gly Pro Ser Glu Val Ile Asn Ser
    890                 895                 900

gct tgc tcc agc tcg ctg gtg gcg ctg cat cgg gcg gtt caa tcg ctg     3989
Ala Cys Ser Ser Ser Leu Val Ala Leu His Arg Ala Val Gln Ser Leu
905                 910                 915                 920

cgc caa ggc gaa agc agt gtc gcc ctg gta ctc ggc gtg aac ctt atc     4037
Arg Gln Gly Glu Ser Ser Val Ala Leu Val Leu Gly Val Asn Leu Ile
                925                 930                 935

ctg gct ccc aag gtg ctg tta gcc agt gca agc gcg ggc atg ctt tcg     4085
Leu Ala Pro Lys Val Leu Leu Ala Ser Ala Ser Ala Gly Met Leu Ser
            940                 945                 950

ccc gat ggc cgc tgc aag acg ctt gac gcc gcc gcc gat ggc ttc gtg     4133
Pro Asp Gly Arg Cys Lys Thr Leu Asp Ala Ala Ala Asp Gly Phe Val
        955                 960                 965

cgt tcg gaa ggg atc gca ggg gtg ata ttg aag cca ctg gcg cag gcg     4181
Arg Ser Glu Gly Ile Ala Gly Val Ile Leu Lys Pro Leu Ala Gln Ala
    970                 975                 980

ctg gcc gat ggt gac agg gtc tac ggt cta gtc cgc ggc gtg gcg gtc     4229
Leu Ala Asp Gly Asp Arg Val Tyr Gly Leu Val Arg Gly Val Ala Val
985                 990                 995                 1000

aac cat ggc ggc cgt  tcc aat tcc ttg cgt  gct ccc aac gtc aac       4274
Asn His Gly Gly Arg  Ser Asn Ser Leu Arg  Ala Pro Asn Val Asn
                1005                1010                1015

gcg cag cgg caa ctg  ctg atc cgg act tac  cag gaa gcc ggt gtc       4319
Ala Gln Arg Gln Leu  Leu Ile Arg Thr Tyr  Gln Glu Ala Gly Val
                1020                1025                1030

gag ccg gcc agc gtc  ggt tat gtt gaa cta  cac ggc act ggt acc       4364
Glu Pro Ala Ser Val  Gly Tyr Val Glu Leu  His Gly Thr Gly Thr
                1035                1040                1045

agc ctg ggt gat ccg  atc gaa atc cag gcg  ctg aag gaa gct ttc       4409
Ser Leu Gly Asp Pro  Ile Glu Ile Gln Ala  Leu Lys Glu Ala Phe
                1050                1055                1060

att gcg ttg ggg gca  cag gcc gcc ccg tca  aac tgc ggc atc ggt       4454
Ile Ala Leu Gly Ala  Gln Ala Ala Pro Ser  Asn Cys Gly Ile Gly
                1065                1070                1075

tcg gtg aag tcc gcg  ctg ggc cat cta gaa  gcc gct gca ggc ctg       4499
Ser Val Lys Ser Ala  Leu Gly His Leu Glu  Ala Ala Ala Gly Leu
                1080                1085                1090

acc ggc ctg atc aag  gtg ctg ctg atg ctc  aag cac ggc gag cag       4544
Thr Gly Leu Ile Lys  Val Leu Leu Met Leu  Lys His Gly Glu Gln
                1095                1100                1105

gcc ggc acg cgc cat  ttc agc acg ctc aat  ccg ctg atc gat ttg       4589
Ala Gly Thr Arg His  Phe Ser Thr Leu Asn  Pro Leu Ile Asp Leu
                1110                1115                1120

cga ggt acg tca ttc  gaa gtg gtg gcg cag  cat cgc gca tgg ccg       4634
Arg Gly Thr Ser Phe  Glu Val Val Ala Gln  His Arg Ala Trp Pro
                1125                1130                1135
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tcg cag gtc ggc att  cac ggc aca ctc ttg  ccg cgt cgc gcg ggt      4679
Ser Gln Val Gly Ile  His Gly Thr Leu Leu  Pro Arg Arg Ala Gly
                1140                1145                1150

atc agc tca ttc ggc  ttc ggc ggc gcc aat  gcg cat gcg atc gtg      4724
Ile Ser Ser Phe Gly  Phe Gly Gly Ala Asn  Ala His Ala Ile Val
                1155                1160                1165

gaa gag cat gtc att  gcc acg ccc ccc tcg  acg agc tcc gct ggc      4769
Glu Glu His Val Ile  Ala Thr Pro Pro Ser  Thr Ser Ser Ala Gly
                1170                1175                1180

ggc ccg gta ggt atc  gtg ttg tca gcc ggt  agt gaa gct gtc ttg      4814
Gly Pro Val Gly Ile  Val Leu Ser Ala Gly  Ser Glu Ala Val Leu
                1185                1190                1195

cgg caa caa gtg ctg  gcc ttg tca gcc tgg  cta agg cag caa tcg      4859
Arg Gln Gln Val Leu  Ala Leu Ser Ala Trp  Leu Arg Gln Gln Ser
                1200                1205                1210

ccg aca ccc gcg caa  atg atc gat gtc gcc  tac acc tta cag gta      4904
Pro Thr Pro Ala Gln  Met Ile Asp Val Ala  Tyr Thr Leu Gln Val
                1215                1220                1225

gga cgc gca gcc ctg  tcg cac agg ttg gct  ttt agc gcg acg gac      4949
Gly Arg Ala Ala Leu  Ser His Arg Leu Ala  Phe Ser Ala Thr Asp
                1230                1235                1240

gcc gag cag gca ttg  gcg agg ctt gag ggt  cgt ctg gcg ggc gtg      4994
Ala Glu Gln Ala Leu  Ala Arg Leu Glu Gly  Arg Leu Ala Gly Val
                1245                1250                1255

atg gat gcc gag gtc  cat cac ggt gtc gtg  gat gct gcc gca acg      5039
Met Asp Ala Glu Val  His His Gly Val Val  Asp Ala Ala Ala Thr
                1260                1265                1270

gct ccc gaa cat ggg  cgg cag acg cgc gaa  ggt ctt gcc ggt ttg      5084
Ala Pro Glu His Gly  Arg Gln Thr Arg Glu  Gly Leu Ala Gly Leu
                1275                1280                1285

ctg cga gcc tgg act  cag ggc gtg cgc gtc  gat tgg tcg gcg ctg      5129
Leu Arg Ala Trp Thr  Gln Gly Val Arg Val  Asp Trp Ser Ala Leu
                1290                1295                1300

tac ggc ata cag cga  ccg cag cgc gtt agc  ctg cct gtc tac ccc      5174
Tyr Gly Ile Gln Arg  Pro Gln Arg Val Ser  Leu Pro Val Tyr Pro
                1305                1310                1315

ttc gct agg gaa cgc  tat tgg ctg ccc ggc  cag gct atg cat gcc      5219
Phe Ala Arg Glu Arg  Tyr Trp Leu Pro Gly  Gln Ala Met His Ala
                1320                1325                1330

gct gcg gac gct cat  ccg atg ctg cag ctg  ttg cat gcc aat gcc      5264
Ala Ala Asp Ala His  Pro Met Leu Gln Leu  Leu His Ala Asn Ala
                1335                1340                1345

aaa cta cat cgc tac  gcc ttg cgt agg tcc  ggc tgc gca agc ttt      5309
Lys Leu His Arg Tyr  Ala Leu Arg Arg Ser  Gly Cys Ala Ser Phe
                1350                1355                1360

ctt gtt gat cat tgc  gtg gat ggt cga cag  gta cta ccg gca gcc      5354
Leu Val Asp His Cys  Val Asp Gly Arg Gln  Val Leu Pro Ala Ala
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                1365                1370                1375

gtg caa ctg gaa ttg  gtg cgc gcc gtg gcg  cag cgg gtc atg gcg      5399
Val Gln Leu Glu Leu  Val Arg Ala Val Ala  Gln Arg Val Met Ala
                1380                1385                1390

cag gat gag ggt tgt  atc gaa ctg gcg cag  gtc gcc ttt ttg cat      5444
Gln Asp Glu Gly Cys  Ile Glu Leu Ala Gln  Val Ala Phe Leu His
                1395                1400                1405

ccc ctc atg atg gag  gag act gag ctg gag  gtc gaa atc gaa ctg      5489
Pro Leu Met Met Glu  Glu Thr Glu Leu Glu  Val Glu Ile Glu Leu
                1410                1415                1420

tcg aag agc gat caa  gat gag ttc gat ttc  caa ctt cac gat gct      5534
Ser Lys Ser Asp Gln  Asp Glu Phe Asp Phe  Gln Leu His Asp Ala
                1425                1430                1435

cac cgc caa cag gtc  ttt agc cag ggg cac  gta cgt cgc cgg gtc      5579
His Arg Gln Gln Val  Phe Ser Gln Gly His  Val Arg Arg Arg Val
                1440                1445                1450

tat acg gcg aca ccg  cgc ttg gat tta gcc  cag ctg caa aag ctt      5624
Tyr Thr Ala Thr Pro  Arg Leu Asp Leu Ala  Gln Leu Gln Lys Leu
                1455                1460                1465

tgt gcc gag cgc gtg  ttg tcc ggc gaa gac  tgt tat gcg cac ttc      5669
Cys Ala Glu Arg Val  Leu Ser Gly Glu Asp  Cys Tyr Ala His Phe
                1470                1475                1480

acc gcc tgc gga ttg  cag ctc ggc gac cgg  ctc aaa tcc gtg caa      5714
Thr Ala Cys Gly Leu  Gln Leu Gly Asp Arg  Leu Lys Ser Val Gln
                1485                1490                1495

tcg atc ggc tgc gga  cgc aat ggc gag ggc  gag ccg atc gca ttg      5759
Ser Ile Gly Cys Gly  Arg Asn Gly Glu Gly  Glu Pro Ile Ala Leu
                1500                1505                1510

ggt gtc ctg cgc ctg  cca cca tca agc gtt  gaa gac agc cat gtg      5804
Gly Val Leu Arg Leu  Pro Pro Ser Ser Val  Glu Asp Ser His Val
                1515                1520                1525

ctg cct cct agc ctg  ctt gat ggt gcc ttg  cag tgt agc ctt ggc      5849
Leu Pro Pro Ser Leu  Leu Asp Gly Ala Leu  Gln Cys Ser Leu Gly
                1530                1535                1540

ttg cag cgt gat gtc  gag cac atc gcc atg  cca tac acg ctg gag      5894
Leu Gln Arg Asp Val  Glu His Ile Ala Met  Pro Tyr Thr Leu Glu
                1545                1550                1555

cgg atg acg gtg cat  gcg ccg att cct ccc  gag gcc tgg gtg ctg      5939
Arg Met Thr Val His  Ala Pro Ile Pro Pro  Glu Ala Trp Val Leu
                1560                1565                1570

ctg cgt cac ggc cat  gca gcc aga cag tcc  ctg gac atc gat ctc      5984
Leu Arg His Gly His  Ala Ala Arg Gln Ser  Leu Asp Ile Asp Leu
                1575                1580                1585

ctg gat tcc gaa ggt  agg gtc tgc gtc agc  ctc ggc aat tac acc      6029
Leu Asp Ser Glu Gly  Arg Val Cys Val Ser  Leu Gly Asn Tyr Thr
                1590                1595                1600

ggc cgt gca ccg aaa  gcc gtt tcc gcc gtc  agg gcg ctt gtc ttg      6074
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Gly Arg Ala Pro Lys  Ala Val Ser Ala Val  Arg Ala Leu Val Leu
                1605                1610                1615

gca ccg gtc tgg caa  gcg ttg acc gaa acg  gcg ccg gca tgg ccc      6119
Ala Pro Val Trp Gln  Ala Leu Thr Glu Thr  Ala Pro Ala Trp Pro
                1620                1625                1630

gat ccg gcc gaa cgc  atc gtt acg gta gga  gac gat gca tgg cgt      6164
Asp Pro Ala Glu Arg  Ile Val Thr Val Gly  Asp Asp Ala Trp Arg
                1635                1640                1645

agt cac ttc ggt ttc  gac gag ccg gcc ttg  tcc ctg gag gac agc      6209
Ser His Phe Gly Phe  Asp Glu Pro Ala Leu  Ser Leu Glu Asp Ser
                1650                1655                1660

gtc gaa gtc atc gcg  acg cga ctg ggc cag  agc ggc aag ttc gat      6254
Val Glu Val Ile Ala  Thr Arg Leu Gly Gln  Ser Gly Lys Phe Asp
                1665                1670                1675

cat cta gtc tgg atc  gtg ccg ata gcc gag  agt gaa acc gat att      6299
His Leu Val Trp Ile  Val Pro Ile Ala Glu  Ser Glu Thr Asp Ile
                1680                1685                1690

gca gcg caa ggt tca  gcg gcg atc gcc ggt  ttc cgg ttg gtc aag      6344
Ala Ala Gln Gly Ser  Ala Ala Ile Ala Gly  Phe Arg Leu Val Lys
                1695                1700                1705

gcg ttg ctt gcg ttg  ggc tat gcg cat cgc  ccg ctg ggt ctc acc      6389
Ala Leu Leu Ala Leu  Gly Tyr Ala His Arg  Pro Leu Gly Leu Thr
                1710                1715                1720

gtg ctg act cgc caa  gcc ctt acg cgg cag  ccg tcg cac gcg gca      6434
Val Leu Thr Arg Gln  Ala Leu Thr Arg Gln  Pro Ser His Ala Ala
                1725                1730                1735

gtg cac ggc ctg atc  ggg acg ccg gcc aag  gaa tac tgc aac tgg      6479
Val His Gly Leu Ile  Gly Thr Leu Ala Lys  Glu Tyr Cys Asn Trp
                1740                1745                1750

aaa atc cgt ctg ctc  gac ctg ccg agc gta  aaa tct tgg ccg caa      6524
Lys Ile Arg Leu Leu  Asp Leu Pro Ser Val  Lys Ser Trp Pro Gln
                1755                1760                1765

tgg gag caa ttg cgg  tcg ttg cct tgg cat  gcg cag ggc gaa gcc      6569
Trp Glu Gln Leu Arg  Ser Leu Pro Trp His  Ala Gln Gly Glu Ala
                1770                1775                1780

ctg atc ggc cgt ggg  act tgt tgg tat cgg  cgg cag ttg tgt gaa      6614
Leu Ile Gly Arg Gly  Thr Cys Trp Tyr Arg  Arg Gln Leu Cys Glu
                1785                1790                1795

gtg ctg ccg ctg ccg  tcg ttg gaa ccg ccg  ccg tac cgc gta ggc      6659
Val Leu Pro Leu Pro  Ser Leu Glu Pro Pro  Pro Tyr Arg Val Gly
                1800                1805                1810

ggt gtc tac gtc gtg  atc ggc ggc gct ggc  ggc ttg ggt gaa gta      6704
Gly Val Tyr Val Val  Ile Gly Gly Ala Gly  Gly Leu Gly Glu Val
                1815                1820                1825

ttg agc gaa cac ttg  atc cgc acg tac gac  gcg cag ctg atc tgg      6749
Leu Ser Glu His Leu  Ile Arg Thr Tyr Asp  Ala Gln Leu Ile Trp
                1830                1835                1840
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atc ggg cgg cgc gtg  ctg gac gaa ggc att  gcg cgc aag cag acc      6794
Ile Gly Arg Arg Val  Leu Asp Glu Gly Ile  Ala Arg Lys Gln Thr
                1845                1850                1855

cgg ctt gcg tcg ctg  ggc cgc gca ccg cat  tac atc tcc gcg gac      6839
Arg Leu Ala Ser Leu  Gly Arg Ala Pro His  Tyr Ile Ser Ala Asp
                1860                1865                1870

gcg agt gac ccg gct  gcc ctg cag gcg gca  cat aat gag atc gtt      6884
Ala Ser Asp Pro Ala  Ala Leu Gln Ala Ala  His Asn Glu Ile Val
                1875                1880                1885

gcg ctg cat ggc cag  ccc cat ggg ctc atc  cta agc aac atc gtg      6929
Ala Leu His Gly Gln  Pro His Gly Leu Ile  Leu Ser Asn Ile Val
                1890                1895                1900

ctg aag gat gcc agt  ctg gct cgt atg gag  gaa gcc gat ttc cgt      6974
Leu Lys Asp Ala Ser  Leu Ala Arg Met Glu  Glu Ala Asp Phe Arg
                1905                1910                1915

gac gtg ctg gcc gcg  aaa ctc gac gtc agc  gtg tgt gcg gca cag      7019
Asp Val Leu Ala Ala  Lys Leu Asp Val Ser  Val Cys Ala Ala Gln
                1920                1925                1930

gtg ttc ggc acg gcc  ccc ctt gat ttc gtg  ctg ttt ttt tct tcc      7064
Val Phe Gly Thr Ala  Pro Leu Asp Phe Val  Leu Phe Phe Ser Ser
                1935                1940                1945

atc cag agc act acc  aag gcg gcc ggg caa  ggt aac tac gcc gcc      7109
Ile Gln Ser Thr Thr  Lys Ala Ala Gly Gln  Gly Asn Tyr Ala Ala
                1950                1955                1960

ggc tgc tgc tat gtc  gac gct ttc ggc gag  cta tgg gcg cgc cgg      7154
Gly Cys Cys Tyr Val  Asp Ala Phe Gly Glu  Leu Trp Ala Arg Arg
                1965                1970                1975

ggt ttg agg gta aag  acc atc aac tgg ggc  tac tgg ggc agc gtg      7199
Gly Leu Arg Val Lys  Thr Ile Asn Trp Gly  Tyr Trp Gly Ser Val
                1980                1985                1990

ggc gtc gta gcg ggc  gag gac tat cgc cgg  cgc atg gcg caa aaa      7244
Gly Val Val Ala Gly  Glu Asp Tyr Arg Arg  Arg Met Ala Gln Lys
                1995                2000                2005

cac atg gct tcg att  gag ggt gcc gaa gcg  atg cag gtg ttg tcg      7289
His Met Ala Ser Ile  Glu Gly Ala Glu Ala  Met Gln Val Leu Ser
                2010                2015                2020
cag ttg ttg tgt gcg  ccg ttg caa cgg ctt  gcc tac gtc aag atc      7334
Gln Leu Leu Cys Ala  Pro Leu Gln Arg Leu  Ala Tyr Val Lys Ile
                2025                2030                2035

gac gat gct aac gca  atg cgc gct ctg ggc  gta gta gag gac gag      7379
Asp Asp Ala Asn Ala  Met Arg Ala Leu Gly  Val Val Glu Asp Glu
                2040                2045                2050

agc gtg caa atc cct  gtg cac gca ccg gcc  gag cct ccc aga ggg      7424
Ser Val Gln Ile Pro  Val His Ala Pro Ala  Glu Pro Pro Arg Gly
                2055                2060                2065

cag cct ggt ccc gtg  gtc gag ttg tcg gtg  aat ctg gat gcc cgg      7469
Gln Pro Gly Pro Val  Val Glu Leu Ser Val  Asn Leu Asp Ala Arg
                2070                2075                2080
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cgc gaa cgg gaa act  ttg ctg gcg gcc tgg  ctg ctt gag ttg atc      7514
Arg Glu Arg Glu Thr  Leu Leu Ala Ala Trp  Leu Leu Glu Leu Ile
                2085                 2090                 2095

gag caa ctc ggt ggt  ttt ccg ccg gca agt  ttc gac atc gct acg      7559
Glu Gln Leu Gly Gly  Phe Pro Pro Ala Ser  Phe Asp Ile Ala Thr
                2100                 2105                 2110

ctt gcg caa cgc ctg  cac atc gta ccc gcc  tat cga agc tgg ctg      7604
Leu Ala Gln Arg Leu  His Ile Val Pro Ala  Tyr Arg Ser Trp Leu
                2115                 2120                 2125

gaa cac agc gtg cgg  atg ctc ggc gtg tat  ggt tac ctc aga gcg      7649
Glu His Ser Val Arg  Met Leu Gly Val Tyr  Gly Tyr Leu Arg Ala
                2130                 2135                 2140

acg ggg gaa agc cga  ttc gag ctg gcc gac  aag ccg ccc gat gat      7694
Thr Gly Glu Ser Arg  Phe Glu Leu Ala Asp  Lys Pro Pro Asp Asp
                2145                 2150                 2155

gcc agg ggt gcc tgg  aac gcg cat gtg cac  gag gcc agc gtc gaa      7739
Ala Arg Gly Ala Trp  Asn Ala His Val His  Glu Ala Ser Val Glu
                2160                 2165                 2170

gcc ggt gaa gag gca  cag cgg cgt ctg ctc  gat cgc tgc atg cgg      7784
Ala Gly Glu Glu Ala  Gln Arg Arg Leu Leu  Asp Arg Cys Met Arg
                2175                 2180                 2185

gcg ttg ccg gcg gtc  ctt cga ggc gaa cgc  aag gcc acc gaa ttg      7829
Ala Leu Pro Ala Val  Leu Arg Gly Glu Arg  Lys Ala Thr Glu Leu
                2190                 2195                 2200

ctg ttt ccg gaa ggt  tcg atg gcg tgg gtc  gag ggt atc tac cag      7874
Leu Phe Pro Glu Gly  Ser Met Ala Trp Val  Glu Gly Ile Tyr Gln
                2205                 2210                 2215

aac aac ccg ctt gcc  gat tac ttc aac gca  caa cta gtc acg cga      7919
Asn Asn Pro Leu Ala  Asp Tyr Phe Asn Ala  Gln Leu Val Thr Arg
                2220                 2225                 2230

ctg att gcc tac ttg  aga cga cga cta gag  tcg acg cct acg gcg      7964
Leu Ile Ala Tyr Leu  Arg Arg Arg Leu Glu  Ser Thr Pro Thr Ala
                2235                 2240                 2245

cgc ctg aag ctg tgc  gag atc ggc gcc ggc  agc ggt ggt act act      8009
Arg Leu Lys Leu Cys  Glu Ile Gly Ala Gly  Ser Gly Gly Thr Thr
                2250                 2255                 2260

gca agc gtg cta caa  cag ttg cag gca tat  ggt gag cat att gag      8054
Ala Ser Val Leu Gln  Gln Leu Gln Ala Tyr  Gly Glu His Ile Glu
                2265                 2270                 2275

gaa tat ctc tat acc  gac ctg tcg cct gtc  ttc ctg cat cat gcg      8099
Glu Tyr Leu Tyr Thr  Asp Leu Ser Pro Val  Phe Leu His His Ala
                2280                 2285                 2290

gaa aaa cac tat cag  cca cga gcg cct tat  ttg agg acc gcc tgt      8144
Glu Lys His Tyr Gln  Pro Arg Ala Pro Tyr  Leu Arg Thr Ala Cys
                2295                 2300                 2305

ttc gac gta gcg cgc  gcg ccg acg gcg cag  gcc ctg gaa tct ggc      8189
Phe Asp Val Ala Arg  Ala Pro Thr Ala Gln  Ala Leu Glu Ser Gly
                2310                 2315                 2320
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ggc tac gac gtg gtg  att gcc gcc aac gta  ctg cat gct acg cgc      8234
Gly Tyr Asp Val Val  Ile Ala Ala Asn Val  Leu His Ala Thr Arg
                2325                2330                2335

gat atc gcc aag acc  ttg cgc aat gcg aag  gca ctc ctc aaa cct      8279
Asp Ile Ala Lys Thr  Leu Arg Asn Ala Lys  Ala Leu Leu Lys Pro
                2340                2345                2350

ggc ggt ctg ctc ttg  ctc aac gaa gtg atc  gag cgc agc ctc gtc      8324
Gly Gly Leu Leu Leu  Leu Asn Glu Val Ile  Glu Arg Ser Leu Val
                2355                2360                2365

ttg cac ctg act ttc  ggt ctg ctg gag agc  tgg tgg ttg ccc cag      8369
Leu His Leu Thr Phe  Gly Leu Leu Glu Ser  Trp Trp Leu Pro Gln
                2370                2375                2380

gac aag atc ttg cgc  ctt gcc ggc tcg ccg  ttg ctg gct tgc gcc      8414
Asp Lys Ile Leu Arg  Leu Ala Gly Ser Pro  Leu Leu Ala Cys Ala
                2385                2390                2395

acc tgg cgc agc ctg  ctg gag gct gag ggt  ttt gcg ggg ctg agc      8459
Thr Trp Arg Ser Leu  Leu Glu Ala Glu Gly  Phe Ala Gly Leu Ser
                2400                2405                2410

gtg cac agg gcg caa  ccc gat gcc ggg cag  gcc atc atc tgt gcc      8504
Val His Arg Ala Gln  Pro Asp Ala Gly Gln  Ala Ile Ile Cys Ala
                2415                2420                2425

tac agc gat ggg ata  gtg cgg caa gcc agt  acg atc gag gtt gcg      8549
Tyr Ser Asp Gly Ile  Val Arg Gln Ala Ser  Thr Ile Glu Val Ala
                2430                2435                2440

cgg aat gaa aaa gta  acc gtt ccg tcg cag  ccg gcg gaa gcc ggg      8594
Arg Asn Glu Lys Val  Thr Val Pro Ser Gln  Pro Ala Glu Ala Gly
                2445                2450                2455

gaa tcg ccg ctg gat  ctg gtc aaa aaa ctg  ctt gga cgc att ctg      8639
Glu Ser Pro Leu Asp  Leu Val Lys Lys Leu  Leu Gly Arg Ile Leu
                2460                2465                2470

aaa atg gat ccg gcc  aca ctc gat acc agc  cac ccg ctg gag tac      8684
Lys Met Asp Pro Ala  Thr Leu Asp Thr Ser  His Pro Leu Glu Tyr
                2475                2480                2485

tac ggt gtc gat tcg  atc gtg gcg atc gaa  ctg gct atg gca ctg      8729
Tyr Gly Val Asp Ser  Ile Val Ala Ile Glu  Leu Ala Met Ala Leu
                2490                2495                2500

cgc gag aca ttc ccg  ggt ttt gaa gtc agc  gag ctg ttt gaa acg      8774
Arg Glu Thr Phe Pro  Gly Phe Glu Val Ser  Glu Leu Phe Glu Thr
                2505                2510                2515

caa tcc atc gat acc  ttg ttg ggc tct ctt  gag cag gct cct ctc      8819
Gln Ser Ile Asp Thr  Leu Leu Gly Ser Leu  Glu Gln Ala Pro Leu
                2520                2525                2530

ctt gct acc ctc aca  gct ccg ccg caa caa  gac atg ctg cag cag      8864
Leu Ala Thr Leu Thr  Ala Pro Pro Gln Gln  Asp Met Leu Gln Gln
                2535                2540                2545

ctg aaa caa ctg ctg  gcg cgt acg ctg aag  ctg gac att acg cag      8909
Leu Lys Gln Leu Leu  Ala Arg Thr Leu Lys  Leu Asp Ile Thr Gln
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                2550                2555                2560

atc gac acg agc aag  acg ctg gag agc tat  ggt gtc gac tcc atc      8954
Ile Asp Thr Ser Lys  Thr Leu Glu Ser Tyr  Gly Val Asp Ser Ile
                2565                2570                2575

gtc atc atc gaa tta  gcc aac gcc ttg cgt  gag cgc tat ccg agc      8999
Val Ile Ile Glu Leu  Ala Asn Ala Leu Arg  Glu Arg Tyr Pro Ser
                2580                2585                2590

ttg gac gcg tca cag  ctg atg gaa acc tta  tcg atc gac cgg ctg      9044
Leu Asp Ala Ser Gln  Leu Met Glu Thr Leu  Ser Ile Asp Arg Leu
                2595                2600                2605

gtt gcc caa tgg cag  gca acg gag ccc gcc  gta ccg gca gag cca      9089
Val Ala Gln Trp Gln  Ala Thr Glu Pro Ala  Val Pro Ala Glu Pro
                2610                2615                2620

aca gcg gaa ccg ccg  gta gcc gac gaa gac  gcc gct gcc atc atc      9134
Thr Ala Glu Pro Pro  Val Ala Asp Glu Asp  Ala Ala Ala Ile Ile
                2625                2630                2635

gga ctg gcc ggc cgc  ttt cca ggc gcg gac  acg ttg gag gag ttc      9179
Gly Leu Ala Gly Arg  Phe Pro Gly Ala Asp  Thr Leu Glu Glu Phe
                2640                2645                2650

tgg aac aac ctg cgc  aac ggc caa agc agt  atg gga gag gtg cca      9224
Trp Asn Asn Leu Arg  Asn Gly Gln Ser Ser  Met Gly Glu Val Pro
                2655                2660                2665

ggc gag cgc tgg gat  cac cag cac tac ttc  gac agt gaa cgc cag      9269
Gly Glu Arg Trp Asp  His Gln His Tyr Phe  Asp Ser Glu Arg Gln
                2670                2675                2680

gca ccg ggc aag acg  tat agc cgc tgg ggt  gcg ttt ctg agg gac      9314
Ala Pro Gly Lys Thr  Tyr Ser Arg Trp Gly  Ala Phe Leu Arg Asp
                2685                2690                2695

ata gac ggc ttc gat  gca gcc ttc ttt gaa  tgg ccc gac agc gtc      9359
Ile Asp Gly Phe Asp  Ala Ala Phe Phe Glu  Trp Pro Asp Ser Val
                2700                2705                2710
gcg ctg gaa tcg gat  ccg caa gcg cgg ata  ttt cta gag cag gcc      9404
Ala Leu Glu Ser Asp  Pro Gln Ala Arg Ile  Phe Leu Glu Gln Ala
                2715                2720                2725

tat gcc ggg atc gaa  gat gcc ggc tac acg  cct ggc tcg ctc agc      9449
Tyr Ala Gly Ile Glu  Asp Ala Gly Tyr Thr  Pro Gly Ser Leu Ser
                2730                2735                2740

aag agc caa cgc gta  ggt gta ttc gta ggt  gtg atg aat ggt tac      9494
Lys Ser Gln Arg Val  Gly Val Phe Val Gly  Val Met Asn Gly Tyr
                2745                2750                2755

tac agc ggc gga gcg  cgc ttc tgg caa atc  gcc aac cgc gtg tcg      9539
Tyr Ser Gly Gly Ala  Arg Phe Trp Gln Ile  Ala Asn Arg Val Ser
                2760                2765                2770

tac cag ttc gat ttt  cgc ggg cca agc ctg  gcg gtg gat acc gcc      9584
Tyr Gln Phe Asp Phe  Arg Gly Pro Ser Leu  Ala Val Asp Thr Ala
                2775                2780                2785

tgt tcg gct tcg ctc  acc gcg atc cac ctg  gcg ctg gaa agc ctg      9629
Cys Ser Ala Ser Leu  Thr Ala Ile His Leu  Ala Leu Glu Ser Leu
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                2790                2795                2800

cgc agc ggc agt tgc  gag gtc gca ctg gcc  ggt ggc gtg aat ctg      9674
Arg Ser Gly Ser Cys  Glu Val Ala Leu Ala  Gly Gly Val Asn Leu
                2805                2810                2815

ctg gtc gat ccg cag  caa tat ctt aat ttg  gct ggc gcc gcg atg      9719
Leu Val Asp Pro Gln  Gln Tyr Leu Asn Leu  Ala Gly Ala Ala Met
                2820                2825                2830

ctc tcc gcc ggc gcc  agc tgt cgg ccg ttc  ggc gag gcc gcg gac      9764
Leu Ser Ala Gly Ala  Ser Cys Arg Pro Phe  Gly Glu Ala Ala Asp
                2835                2840                2845

ggt ttc gtg gcc ggc  gaa gcc tgc ggc gtg  gtg ctg ctc aag ccg      9809
Gly Phe Val Ala Gly  Glu Ala Cys Gly Val  Val Leu Leu Lys Pro
                2850                2855                2860

ctc aag caa gcg agg  gcc gat ggc gat gtg  atc cat gcc gta atc      9854
Leu Lys Gln Ala Arg  Ala Asp Gly Asp Val  Ile His Ala Val Ile
                2865                2870                2875

agg ggc agc atg atc  aat gcc ggt ggg cac  acc agc gcg ttc tcc      9899
Arg Gly Ser Met Ile  Asn Ala Gly Gly His  Thr Ser Ala Phe Ser
                2880                2885                2890

tcg cct aac cct gcc  gcc cag gcc gaa gtc  gtg cgg cag gcc ttg      9944
Ser Pro Asn Pro Ala  Ala Gln Ala Glu Val  Val Arg Gln Ala Leu
                2895                2900                2905

cag cgc gcg ggc gtg  gcg ccc gat tcg atc  agc tac atc gag gcg      9989
Gln Arg Ala Gly Val  Ala Pro Asp Ser Ile  Ser Tyr Ile Glu Ala
                2910                2915                2920

cat ggc acc ggc acc  gta cta ggc gat gca  gtg gag ttg ggt gct     10034
His Gly Thr Gly Thr  Val Leu Gly Asp Ala  Val Glu Leu Gly Ala
                2925                2930                2935

ttg aat aaa gtg ttc  gac aag cgc gcg gcg  cca tgc ccg atc ggc     10079
Leu Asn Lys Val Phe  Asp Lys Arg Ala Ala  Pro Cys Pro Ile Gly
                2940                2945                2950

tcg ctg aag gcg aac  atc ggc cat gcc gaa  agc gcc gcg ggc atc     10124
Ser Leu Lys Ala Asn  Ile Gly His Ala Glu  Ser Ala Ala Gly Ile
                2955                2960                2965

gcc ggc ctg gcc aag  ctg gta ttg cag ttc  agg cat ggc gag ttg     10169
Ala Gly Leu Ala Lys  Leu Val Leu Gln Phe  Arg His Gly Glu Leu
                2970                2975                2980

gtg cct agt ctg aat  gcg ttt ccc ttg aat  ccc tat att gag ttc     10214
Val Pro Ser Leu Asn  Ala Phe Pro Leu Asn  Pro Tyr Ile Glu Phe
                2985                2990                2995

ggt cgc ttc cag gta  caa cag cag ccg gca  ccg tgg ccg cgc cgt     10259
Gly Arg Phe Gln Val  Gln Gln Gln Pro Ala  Pro Trp Pro Arg Arg
                3000                3005                3010

ggc gcc cag ccg cgg  cgc gcc ggg tta tct  gcc ttc ggt gct ggc     10304
Gly Ala Gln Pro Arg  Arg Ala Gly Leu Ser  Ala Phe Gly Ala Gly
                3015                3020                3025

gga tcg aat gcg cac  cta gtg gta gag gaa  gct ccg gct atg gct     10349
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Gly Ser Asn Ala His  Leu Val Val Glu Glu  Ala Pro Ala Met Ala
                3030                3035                3040

ccc ggg gtc tcg atc  agc gcc agc tct cca  gcc ttg atc gtg ctt      10394
Pro Gly Val Ser Ile  Ser Ala Ser Ser Pro  Ala Leu Ile Val Leu
                3045                3050                3055

tcg gcg cga acg ctg  cct gcc ttg caa cag  cgt gct cgc gat ctg      10439
Ser Ala Arg Thr Leu  Pro Ala Leu Gln Gln  Arg Ala Arg Asp Leu
                3060                3065                3070

ctc gtc tgg atg caa  gcg cgg cag gtg gat  gac gtc atg ctg gcc      10484
Leu Val Trp Met Gln  Ala Arg Gln Val Asp  Asp Val Met Leu Ala
                3075                3080                3085

gac gtt gct tat acg  ctg cac ttg ggc cgc  gtc gcg atg gag caa      10529
Asp Val Ala Tyr Thr  Leu His Leu Gly Arg  Val Ala Met Glu Gln
                3090                3095                3100

cgc ctg gct ttt acc  gct ggc tcg gct gcc  gag ttg agc gag aaa      10574
Arg Leu Ala Phe Thr  Ala Gly Ser Ala Ala  Glu Leu Ser Glu Lys
                3105                3110                3115

tta cag gct tac ctg  ggc cat gcg att cgg  gcc gac atc tat ctg      10619
Leu Gln Ala Tyr Leu  Gly His Ala Ile Arg  Ala Asp Ile Tyr Leu
                3120                3125                3130

agc gag gac acg ccc  ggc aaa ccg gca ggc  gct ccg atc gtg gcc      10664
Ser Glu Asp Thr Pro  Gly Lys Pro Ala Gly  Ala Pro Ile Val Ala
                3135                3140                3145

gag gaa gat ctg ctc  acg ctg atg gat gcc  tgg atc gaa aag ggc      10709
Glu Glu Asp Leu Leu  Thr Leu Met Asp Ala  Trp Ile Glu Lys Gly
                3150                3155                3160

cag tac ggt cgt ttg  ctg gag tac tgg acc  aag ggc caa ccg atc      10754
Gln Tyr Gly Arg Leu  Leu Glu Tyr Trp Thr  Lys Gly Gln Pro Ile
                3165                3170                3175

gac tgg aac aaa ctc  tat tgg cgc aag ctg  tat gcg gac gga cgg      10799
Asp Trp Asn Lys Leu  Tyr Trp Arg Lys Leu  Tyr Ala Asp Gly Arg
                3180                3185                3190

ccg cgg cgg atc agc  ctg ccc acc tat ccg  ttc gag cac cgg cgt      10844
Pro Arg Arg Ile Ser  Leu Pro Thr Tyr Pro  Phe Glu His Arg Arg
                3195                3200                3205

tat tgg caa acg ccg  gtg ccg ggc gag cga  agc ctg cac gcc acc      10889
Tyr Trp Gln Thr Pro  Val Pro Gly Glu Arg  Ser Leu His Ala Thr
                3210                3215                3220

gcg cca gct act cgg  gaa acg gtt gcg gtt  ggt gcc atg ccg gat      10934
Ala Pro Ala Thr Arg  Glu Thr Val Ala Val  Gly Ala Met Pro Asp
                3225                3230                3235

ccg gcc ggc gct acg  gtg caa gcc cgg ttg  tgc gcc ttg tgc caa      10979
Pro Ala Gly Ala Thr  Val Gln Ala Arg Leu  Cys Ala Leu Cys Gln
                3240                3245                3250

gtg ttg ttg ggc aaa  ccg gtc acg gcc cag  atg gat ttc ttt gcc      11024
Val Leu Leu Gly Lys  Pro Val Thr Ala Gln  Met Asp Phe Phe Ala
                3255                3260                3265
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gtc ggc ggc cat tcg  gtg ctg gcg atc caa  ttg gtc tcg cgc atc      11069
Val Gly Gly His Ser  Val Leu Ala Ile Gln  Leu Val Ser Arg Ile
                3270                3275                3280

cgc aaa agc ttc ggg  gtg gag tat ccg gtc  agc gct ttg ttc gaa      11114
Arg Lys Ser Phe Gly  Val Glu Tyr Pro Val  Ser Ala Leu Phe Glu
                3285                3290                3295

tcg gcg ctg ttg tcg  gac atg gcg cgg cag  atc gaa caa ttg cgg      11159
Ser Ala Leu Leu Ser  Asp Met Ala Arg Gln  Ile Glu Gln Leu Arg
                3300                3305                3310

gtg aac gga gtc gcc  aag cgc atg ccg gcg  ttg ttg cct gcc ggg      11204
Val Asn Gly Val Ala  Lys Arg Met Pro Ala  Leu Leu Pro Ala Gly
                3315                3320                3325

cgc gtg ggc gcg att  cct gcg act tat gca  cag gag cgc cta tgg      11249
Arg Val Gly Ala Ile  Pro Ala Thr Tyr Ala  Gln Glu Arg Leu Trp
                3330                3335                3340

ctc gtc cac gaa cat  atg agt gag caa cgc  agt agt tac aac atc      11294
Leu Val His Glu His  Met Ser Glu Gln Arg  Ser Ser Tyr Asn Ile
                3345                3350                3355

acc ttt gcc atg cac  ttc aga ggc gtc gac  ttc cgt gct gaa gcg      11339
Thr Phe Ala Met His  Phe Arg Gly Val Asp  Phe Arg Ala Glu Ala
                3360                3365                3370

atg cgt gcc gca ttg  aac gcg ctg gtg gtg  cgg cac gaa gtg ctg      11384
Met Arg Ala Ala Leu  Asn Ala Leu Val Val  Arg His Glu Val Leu
                3375                3380                3385

cgc aca cgc ttt ctt  tcg gag gac ggg cag  ctg caa cag gtg atc      11429
Arg Thr Arg Phe Leu  Ser Glu Asp Gly Gln  Leu Gln Gln Val Ile
                3390                3395                3400

gct gct tcg ttg acg  ttg gag gtg ccg gta  aga gag atg tcg gtc      11474
Ala Ala Ser Leu Thr  Leu Glu Val Pro Val  Arg Glu Met Ser Val
                3405                3410                3415

gag gag gtc gac ctg  ctg ctg gcc gcg agc  acg cgg gag act ttc      11519
Glu Glu Val Asp Leu  Leu Leu Ala Ala Ser  Thr Arg Glu Thr Phe
                3420                3425                3430

gat ctg cgg cag ggg  ccc ttg ttc aag gca  cgc atc ctg cgc gtg      11564
Asp Leu Arg Gln Gly  Pro Leu Phe Lys Ala  Arg Ile Leu Arg Val
                3435                3440                3445

gcg gcc gat cac cat  gtg gtg ttg agc agc  atc cac cac atc att      11609
Ala Ala Asp His His  Val Val Leu Ser Ser  Ile His His Ile Ile
                3450                3455                3460

tcc gac ggc tgg tcg  ctg gga gtg ttc aac  cgt gac ctg cac cag      11654
Ser Asp Gly Trp Ser  Leu Gly Val Phe Asn  Arg Asp Leu His Gln
                3465                3470                3475

ctg tac gag gcg tgt  ttg cgc ggc acg ccc  ccc aca ctg ccg acg      11699
Leu Tyr Glu Ala Cys  Leu Arg Gly Thr Pro  Pro Thr Leu Pro Thr
                3480                3485                3490

ctg gcg gtg cag tat  gcc gac tac gcg ctg  tgg caa cgg caa tgg      11744
Leu Ala Val Gln Tyr  Ala Asp Tyr Ala Leu  Trp Gln Arg Gln Trp
                3495                3500                3505
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gag ctg gcg gct ccg  ctg tcg tac tgg acg  cgg gca ctg gaa ggc      11789
Glu Leu Ala Ala Pro  Leu Ser Tyr Trp Thr  Arg Ala Leu Glu Gly
                3510                3515                3520

tac gac gac ggc ctg  gac ttg ccc tac gac  cgg ccg cgc ggc gcc      11834
Tyr Asp Asp Gly Leu  Asp Leu Pro Tyr Asp  Arg Pro Arg Gly Ala
                3525                3530                3535

acg cgg gcg tgg cgg  gca ggg ctg gtc aaa  cac cgc tat ccg ccg      11879
Thr Arg Ala Trp Arg  Ala Gly Leu Val Lys  His Arg Tyr Pro Pro
                3540                3545                3550

caa ctg gcc cag cag  ttg gcg gcc tac agc  caa cag tac caa gcg      11924
Gln Leu Ala Gln Gln  Leu Ala Ala Tyr Ser  Gln Gln Tyr Gln Ala
                3555                3560                3565

acg ctg ttc atg agc  ctg ctg gca ggc ctg  gcg ttg gtg ctg ggc      11969
Thr Leu Phe Met Ser  Leu Leu Ala Gly Leu  Ala Leu Val Leu Gly
                3570                3575                3580

cgt tac gcc gat cgc  aag gac gtg tgc atc  ggc gcg acg gtc tcc      12014
Arg Tyr Ala Asp Arg  Lys Asp Val Cys Ile  Gly Ala Thr Val Ser
                3585                3590                3595

ggc cgc gac cag ctg  gag ctg gaa gag ctg  atc ggc ttt ttc atc      12059
Gly Arg Asp Gln Leu  Glu Leu Glu Glu Leu  Ile Gly Phe Phe Ile
                3600                3605                3610

aat att ttg ccg ctg  cgg gtg gac ctg tcg  ggg gat ccg tgc ctg      12104
Asn Ile Leu Pro Leu  Arg Val Asp Leu Ser  Gly Asp Pro Cys Leu
                3615                3620                3625

gag gag gtg ctg ctg  cgc acg cgt caa gtg  gta ctg gat ggc ttc      12149
Glu Glu Val Leu Leu  Arg Thr Arg Gln Val  Val Leu Asp Gly Phe
                3630                3635                3640

gcg cac cag tcg gtg  ccg ttc gag cac gtg  ttg cag gcg ctg cgg      12194
Ala His Gln Ser Val  Pro Phe Glu His Val  Leu Gln Ala Leu Arg
                3645                3650                3655

cgt cag cgc gac agt  agc cag atc ccg ctg  gtg ccg gtg atg ctg      12239
Arg Gln Arg Asp Ser  Ser Gln Ile Pro Leu  Val Pro Val Met Leu
                3660                3665                3670

cga cac cag aac ttc  ccg acg cag gag att  ggc gat tgg ccc gag      12284
Arg His Gln Asn Phe  Pro Thr Gln Glu Ile  Gly Asp Trp Pro Glu
                3675                3680                3685

gga gtg cgg ctg acg  cag atg gag ctg ggg  ctg gac cgt agc acg      12329
Gly Val Arg Leu Thr  Gln Met Glu Leu Gly  Leu Asp Arg Ser Thr
                3690                3695                3700

ccg agc gag ctg gat  tgg cag ttc tac ggc  gac ggc agc tcg ctg      12374
Pro Ser Glu Leu Asp  Trp Gln Phe Tyr Gly  Asp Gly Ser Ser Leu
                3705                3710                3715

gag ctg acg ctg gaa  tac gcg cag gac ctc  ttc gac gaa gcg acg      12419
Glu Leu Thr Leu Glu  Tyr Ala Gln Asp Leu  Phe Asp Glu Ala Thr
                3720                3725                3730

gtg cgg cgg atg atc  gca cac cac cag cag  gcg ttg gag gcg atg      12464
Val Arg Arg Met Ile  Ala His His Gln Gln  Ala Leu Glu Ala Met
                3735                3740                3745
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gtg agc cgg cca cag  ctg cgg gtg ggc aag  tgg gac atg ctg acg      12509
Val Ser Arg Pro Gln  Leu Arg Val Gly Lys  Trp Asp Met Leu Thr
                3750                 3755                 3760

gcc gaa gag cgc cgg  ctg ttt gcc gcg cta  aat gcg aca ggt acg      12554
Ala Glu Glu Arg Arg  Leu Phe Ala Ala Leu  Asn Ala Thr Gly Thr
                3765                 3770                 3775

cca cgg gag tgg ccc  agt ctg gcg cag cag  ttc gaa cgg cag gcg      12599
Pro Arg Glu Trp Pro  Ser Leu Ala Gln Gln  Phe Glu Arg Gln Ala
                3780                 3785                 3790

cag gcg acg ccg cag  gcc ata gca tgc gtg  agc gat ggg cag tcg      12644
Gln Ala Thr Pro Gln  Ala Ile Ala Cys Val  Ser Asp Gly Gln Ser
                3795                 3800                 3805

tgg agc tat gcg cag  ttg gag gcg cgc gcc  aac cag ctg gca cag      12689
Trp Ser Tyr Ala Gln  Leu Glu Ala Arg Ala  Asn Gln Leu Ala Gln
                3810                 3815                 3820

gcg ctg cgt ggg cag  ggc gcg ggc cgg gac  gtg cgg gtg gcg gta      12734
Ala Leu Arg Gly Gln  Gly Ala Gly Arg Asp  Val Arg Val Ala Val
                3825                 3830                 3835

cag agt gcg cgc acg  ccg gaa ctg ctg atg  gcc ttg ctg gcg atc      12779
Gln Ser Ala Arg Thr  Pro Glu Leu Leu Met  Ala Leu Leu Ala Ile
                3840                 3845                 3850

ttc aag gcc ggt gca  tgc tat gtg ccg atc  gat ccg gcc tac ccg      12824
Phe Lys Ala Gly Ala  Cys Tyr Val Pro Ile  Asp Pro Ala Tyr Pro
                3855                 3860                 3865

gcg gcc tac cgc gag  cag atc ctg gcc gag  gtg cag gtg tcg atc      12869
Ala Ala Tyr Arg Glu  Gln Ile Leu Ala Glu  Val Gln Val Ser Ile
                3870                 3875                 3880

gtg ctg gag caa gac  gag ctg gcg ctg gac  gag caa ggg cag ttc      12914
Val Leu Glu Gln Asp  Glu Leu Ala Leu Asp  Glu Gln Gly Gln Phe
                3885                 3890                 3895

cac aat ccg cgt tgg  cgc gag caa gcc ccg  acg ccg ctg ggg ctg      12959
His Asn Pro Arg Trp  Arg Glu Gln Ala Pro  Thr Pro Leu Gly Leu
                3900                 3905                 3910

agg gaa cat ccg ggc  gac ctg gcg tgc gtg  atg gtg acc tcc ggc      13004
Arg Glu His Pro Gly  Asp Leu Ala Cys Val  Met Val Thr Ser Gly
                3915                 3920                 3925

tcg acc ggc cgg ccc  aag ggc gtg atg gtg  ccg tat gcg cag ctg      13049
Ser Thr Gly Arg Pro  Lys Gly Val Met Val  Pro Tyr Ala Gln Leu
                3930                 3935                 3940

cac aac tgg ctg cat  gca ggc tgg cag cgt  tct gcg ttc gag gcc      13094
His Asn Trp Leu His  Ala Gly Trp Gln Arg  Ser Ala Phe Glu Ala
                3945                 3950                 3955

ggg gag cgg gtg ctg  cag aag acc tcg atc  gcc ttt gcg gtg tcg      13139
Gly Glu Arg Val Leu  Gln Lys Thr Ser Ile  Ala Phe Ala Val Ser
                3960                 3965                 3970

gta aag gag ttg cta  agc ggg ctg ctg gcg  ggg gtg gaa cag gtg      13184
Val Lys Glu Leu Leu  Ser Gly Leu Leu Ala  Gly Val Glu Gln Val
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                3975                3980                3985

atg ctg ccg gac gag  cag gtg aag gac agc  ctg gcg ttg gcg cgg      13229
Met Leu Pro Asp Glu  Gln Val Lys Asp Ser  Leu Ala Leu Ala Arg
                3990                3995                4000

gcg att gag caa tgg  cag gtg acg cgg ctg  tac cta gtg cca tcg      13274
Ala Ile Glu Gln Trp  Gln Val Thr Arg Leu  Tyr Leu Val Pro Ser
                4005                4010                4015

cac ctg cag gcg ctg  ctg gac gcg acg caa  gga cga gac ggg cta      13319
His Leu Gln Ala Leu  Leu Asp Ala Thr Gln  Gly Arg Asp Gly Leu
                4020                4025                4030

ctg cac tcg ctg cgt  cac gtg gtg acg gcg  ggg gaa gcg ttg ccg      13364
Leu His Ser Leu Arg  His Val Val Thr Ala  Gly Glu Ala Leu Pro
                4035                4040                4045

tct gcg gtg cgc gaa  acg gtg cgg gtg cgt  ctg cca cag gtg cag      13409
Ser Ala Val Arg Glu  Thr Val Arg Val Arg  Leu Pro Gln Val Gln
                4050                4055                4060

cta tgg aac aac tat  ggc tgc acg gaa ctg  aac gac gcg acc tac      13454
Leu Trp Asn Asn Tyr  Gly Cys Thr Glu Leu  Asn Asp Ala Thr Tyr
                4065                4070                4075

cat cgg tcg gat acg  gtg gcg cca gga acg  ttt gtg ccg atc ggc      13499
His Arg Ser Asp Thr  Val Ala Pro Gly Thr  Phe Val Pro Ile Gly
                4080                4085                4090
gca ccg atc gcc aac  acc gag gta tac gtg  ctg gac cgg cag ctg      13544
Ala Pro Ile Ala Asn  Thr Glu Val Tyr Val  Leu Asp Arg Gln Leu
                4095                4100                4105

cgg cag gtg ccg atc  ggg gtg atg ggc gag  ctg cac gta cac agc      13589
Arg Gln Val Pro Ile  Gly Val Met Gly Glu  Leu His Val His Ser
                4110                4115                4120

gtg ggg atg gcg cgc  ggc tac tgg aac cgg  ccg ggg ctg acg gcc      13634
Val Gly Met Ala Arg  Gly Tyr Trp Asn Arg  Pro Gly Leu Thr Ala
                4125                4130                4135

tcg cgc ttc atc gcg  cac ccg tat agc gag  gag ccg ggc aca cgg      13679
Ser Arg Phe Ile Ala  His Pro Tyr Ser Glu  Glu Pro Gly Thr Arg
                4140                4145                4150

ctg tac aag acc ggt  gac atg gta cgc cgg  ctg gcg gac ggg acg      13724
Leu Tyr Lys Thr Gly  Asp Met Val Arg Arg  Leu Ala Asp Gly Thr
                4155                4160                4165

ctg gaa tac ctg ggc  cga cag gac ttc gag  gtc aag gtg cgc ggc      13769
Leu Glu Tyr Leu Gly  Arg Gln Asp Phe Glu  Val Lys Val Arg Gly
                4170                4175                4180

cac cgg gtg gat acg  cgg cag gtg gag gcg  gcc ttg cgg gcg cag      13814
His Arg Val Asp Thr  Arg Gln Val Glu Ala  Ala Leu Arg Ala Gln
                4185                4190                4195

ccc gcg gtg gcc gag  gcg gtg gtg agc ggt  cac cgg gtg gac ggg      13859
Pro Ala Val Ala Glu  Ala Val Val Ser Gly  His Arg Val Asp Gly
                4200                4205                4210

gac atg cag ttg gtg  gcc tat gtg gtg gcg  cgt gaa ggg cag gca      13904
Asp Met Gln Leu Val  Ala Tyr Val Val Ala  Arg Glu Gly Gln Ala
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ccg agc gcg ggc gag  ttg aaa caa cag ctg  tcg gcg cag ttg ccg      13949
Pro Ser Ala Gly Glu  Leu Lys Gln Gln Leu  Ser Ala Gln Leu Pro
                4230                4235                4240

acc tac atg ctg ccg  acc gtg tac cag tgg  ctg gag cag ttg ccg      13994
Thr Tyr Met Leu Pro  Thr Val Tyr Gln Trp  Leu Glu Gln Leu Pro
                4245                4250                4255

cgg ctg tcc aac ggc  aag ttg gac cgg ttg  gcg ctg ccg gcg ccg      14039
Arg Leu Ser Asn Gly  Lys Leu Asp Arg Leu  Ala Leu Pro Ala Pro
                4260                4265                4270

cag gtg gta cac gcg  cag gag tac gtc gcg  cca cgc aac gag gcc      14084
Gln Val Val His Ala  Gln Glu Tyr Val Ala  Pro Arg Asn Glu Ala
                4275                4280                4285

gag caa cgg ctg gcg  gca ctg ttt gcc gag  gtg ctg cgg gtg gag      14129
Glu Gln Arg Leu Ala  Ala Leu Phe Ala Glu  Val Leu Arg Val Glu
                4290                4295                4300

cag gtg ggc atc cac  gac aac ttc ttc gcc  ttg ggt ggg cac tcg      14174
Gln Val Gly Ile His  Asp Asn Phe Phe Ala  Leu Gly Gly His Ser
                4305                4310                4315

ctg tct gca tcg caa  ctg atc tcg cgc atc  cgc caa agt ttt cac      14219
Leu Ser Ala Ser Gln  Leu Ile Ser Arg Ile  Arg Gln Ser Phe His
                4320                4325                4330

gtc gat ctg ccg ctg  agc cgg atc ttc gag  gca ccc acg atc gag      14264
Val Asp Leu Pro Leu  Ser Arg Ile Phe Glu  Ala Pro Thr Ile Glu
                4335                4340                4345

ggc ctg gtc agg cag  cta gcg ttg cct agt  gaa ggc ggc gtg gcc      14309
Gly Leu Val Arg Gln  Leu Ala Leu Pro Ser  Glu Gly Gly Val Ala
                4350                4355                4360

agc atc gcc agg gta  gcg cga aac cgg acg  atc cca ttg tcg ctg      14354
Ser Ile Ala Arg Val  Ala Arg Asn Arg Thr  Ile Pro Leu Ser Leu
                4365                4370                4375

ttc cag gaa cgc ctg  tgg ttc gtg cac caa  cac atg cct gag caa      14399
Phe Gln Glu Arg Leu  Trp Phe Val His Gln  His Met Pro Glu Gln
                4380                4385                4390

cgc acc agt tac aac  ggc acg ctc gcc ttg  cgt ttg cgt ggt cct      14444
Arg Thr Ser Tyr Asn  Gly Thr Leu Ala Leu  Arg Leu Arg Gly Pro
                4395                4400                4405

ttg tcg gtg gaa gcg  atg cgt gca gcg ctg  cgt gcg tta gtg ctg      14489
Leu Ser Val Glu Ala  Met Arg Ala Ala Leu  Arg Ala Leu Val Leu
                4410                4415                4420

cgc cac gaa atc ttg  cgt acc cgc ttc gtg  ttg ccg acc ggt gct      14534
Arg His Glu Ile Leu  Arg Thr Arg Phe Val  Leu Pro Thr Gly Ala
                4425                4430                4435

agc gag ccg gtg cag  gtc att gac gag cac  agc gat ttc cag ctc      14579
Ser Glu Pro Val Gln  Val Ile Asp Glu His  Ser Asp Phe Gln Leu
                4440                4445                4450

tca gta cag cta gtc  gag gat act gag atc  gcg tcg ctg atg gat      14624
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Ser Val Gln Leu Val  Glu Asp Thr Glu Ile  Ala Ser Leu Met Asp
                4455                4460                4465

gaa ctg gca agt cat  atc tac gac tta gcc  aac ggc ccg ctg ttc      14669
Glu Leu Ala Ser His  Ile Tyr Asp Leu Ala  Asn Gly Pro Leu Phe
                4470                4475                4480

att gca tgc ctt ttg  caa ctg gat gag caa  gaa cat gtg ctg cta      14714
Ile Ala Cys Leu Leu  Gln Leu Asp Glu Gln  Glu His Val Leu Leu
                4485                4490                4495

atc ggc atg cat cac  ctt atc tac gac gct  tgg tcg caa ttc acc      14759
Ile Gly Met His His  Leu Ile Tyr Asp Ala  Trp Ser Gln Phe Thr
                4500                4505                4510

gtg atg aac cgc gat  cta cgc gtg ctg tat  cac cgc cac ctc gga      14804
Val Met Asn Arg Asp  Leu Arg Val Leu Tyr  His Arg His Leu Gly
                4515                4520                4525

ctt gcc ggc gga gat  ctg ccg gaa tta ccg  atc caa tat gcc gac      14849
Leu Ala Gly Gly Asp  Leu Pro Glu Leu Pro  Ile Gln Tyr Ala Asp
                4530                4535                4540

tat gcg atc tgg caa  cgc gcc cag aac ctg  gac gcg caa ctg gcc      14894
Tyr Ala Ile Trp Gln  Arg Ala Gln Asn Leu  Asp Ala Gln Leu Ala
                4545                4550                4555

tat tgg cag gct atg  ttg cac gac tac gac  gac ggc ctg gag ctg      14939
Tyr Trp Gln Ala Met  Leu His Asp Tyr Asp  Asp Gly Leu Glu Leu
                4560                4565                4570

ccc tac gac tat ccg  cgt ccg cgc aat cgc  acc tgg cac gca gcg      14984
Pro Tyr Asp Tyr Pro  Arg Pro Arg Asn Arg  Thr Trp His Ala Ala
                4575                4580                4585

gtc tac acc cac acc  tat ccg gct gaa ctg  gtc cag cgc ttt gcc      15029
Val Tyr Thr His Thr  Tyr Pro Ala Glu Leu  Val Gln Arg Phe Ala
                4590                4595                4600

ggc ttc gta cag gcg  cat cag tcg acc ttg  ttc atc ggg ctg ttg      15074
Gly Phe Val Gln Ala  His Gln Ser Thr Leu  Phe Ile Gly Leu Leu
                4605                4610                4615

gcc agc ttc gcg gtc  gtg ttg aac aaa tac  acc ggc cgg gac gac      15119
Ala Ser Phe Ala Val  Val Leu Asn Lys Tyr  Thr Gly Arg Asp Asp
                4620                4625                4630

ttg tgc atc ggt acc  acc acg gca ggg cgc  acg cac ctg gag ctg      15164
Leu Cys Ile Gly Thr  Thr Thr Ala Gly Arg  Thr His Leu Glu Leu
                4635                4640                4645

gag aac ctg atc ggt  ttc ttc atc aac atc  ttg cct ttg cgc ttg      15209
Glu Asn Leu Ile Gly  Phe Phe Ile Asn Ile  Leu Pro Leu Arg Leu
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cgc ttg gac ggc gat  ccg gac gtt gcc gaa  atc atg cgg cga aca      15254
Arg Leu Asp Gly Asp  Pro Asp Val Ala Glu  Ile Met Arg Arg Thr
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cgg ttg gtg gcg atg  agc gcg ttt gag aac  cag gcg cta ccg ttc      15299
Arg Leu Val Ala Met  Ser Ala Phe Glu Asn  Gln Ala Leu Pro Phe
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gag cac ctg ctc aac  gcc ctg cac aag caa  cgt gac acc agc cgg      15344
Glu His Leu Leu Asn  Ala Leu His Lys Gln  Arg Asp Thr Ser Arg
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att ccg cta gtt ccg  gtg gtg atg cgt cat  cag aac ttc ccg gac      15389
Ile Pro Leu Val Pro  Val Val Met Arg His  Gln Asn Phe Pro Asp
                4710                4715                4720

acg atc ggc gac tgg  agc gat ggc atc cgt  acc gaa gtg atc cag      15434
Thr Ile Gly Asp Trp  Ser Asp Gly Ile Arg  Thr Glu Val Ile Gln
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cgc gat ctg cgt gcc  acc ccc aat gaa atg  gac ctg caa ttc ttc      15479
Arg Asp Leu Arg Ala  Thr Pro Asn Glu Met  Asp Leu Gln Phe Phe
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ggc gac ggt acg ggg  ctt tcg gtc aca gtg  gaa tac gcg gcg gag      15524
Gly Asp Gly Thr Gly  Leu Ser Val Thr Val  Glu Tyr Ala Ala Glu
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ctg ttc tca gaa gcg  acc att cgc cgc ctg  atc cac cat cac caa      15569
Leu Phe Ser Glu Ala  Thr Ile Arg Arg Leu  Ile His His His Gln
                4770                4775                4780
ctc gtc ctg gag cag  atg ttg gcg gcc cat  gaa agc gcc acg tgc      15614
Leu Val Leu Glu Gln  Met Leu Ala Ala His  Glu Ser Ala Thr Cys
                4785                4790                4795

ccc ttg gat gtt gcc  gac tagcaaaagc cggccgccgt cacccgttca          15662
Pro Leu Asp Val Ala  Asp
                4800

tcgatagcga gggcaatcat ggattcagcg ttacctacat ctgcatttac cttcgatctc  15722

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<212> PRT
<213> Xanthomonas albilineans

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Gly Met Arg Arg Ala Trp Pro Pro Phe Pro Gln Ala Cys Cys Arg Ser
35 40 45

Ile Ala Tyr Leu Met Gln Arg Ser Pro Met Ser Pro Leu Gln Gln Thr
50 55 60

Leu Leu Thr Arg Leu Ala Ser Ala Ala Ala Ser Arg Thr Met Ile Glu
65 70 75 80

Phe Pro Arg Pro Glu His Ala Ser Pro Gln Cys Cys Asp Asp Ala Glu
85 90 95

Leu Ala Arg Leu Ile Val Gln Leu Ser Ala Gly Leu Gln Pro Leu Ala
100 105 110

Met Pro Gly Thr Tyr Val Ile Ile Ala Ala Pro His Gly Gly Leu Phe
115 120 125

Ala Ala Ala Leu Leu Ala Cys Leu His Ala Asn Leu Val Ala Val Pro
130 135 140

Phe Pro Leu Asp Val Ala Gln Pro Asn Glu Arg Glu Gln Ala Arg Leu
145 150 155 160

Glu Thr Ile His Ala Gln Leu Met Glu His Gly Asn Val Ala Val Leu
165 170 175

Leu Asp Asp Val Ala Asp Arg Ser Ala Phe Ala Arg Met Ala His Ala
180 185 190

Ala Gly Thr Phe Leu Ala Thr Phe Ala Asp Leu Lys Arg Glu Ser Thr
195 200 205

Ser Ala Ser Leu Cys Pro Ala Ser Pro Ser Asp Ala Ala Leu Leu Leu
210 215 220

Phe Thr Ser Gly Ser Ser Gly Glu Ser Lys Gly Ile Leu Leu Ser His
225 230 235 240

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Arg Asn Leu His His Gln Ile Gln Ala Gly Ile Arg Gln Trp Ser Leu
                245                 250                 255

Asp Glu His Ser His Val Val Thr Trp Leu Ser Pro Ala His Asn Phe
            260                 265                 270

Gly Leu His Phe Gly Leu Leu Ala Pro Trp Phe Ser Gly Ala Thr Val
        275                 280                 285

Ser Phe Ile His Pro His Ser Tyr Met Lys Arg Pro Gly Phe Trp Leu
    290                 295                 300

Glu Thr Val Ala Ala Arg Asp Ala Thr His Met Ala Ala Pro Asn Phe
305                 310                 315                 320

Ala Phe Asp Tyr Cys Cys Asp Trp Val Met Val Glu Gln Leu Pro Pro
                325                 330                 335

Ser Ala Leu Ser Thr Leu Thr His Ile Val Cys Gly Gly Glu Pro Val
            340                 345                 350

Arg Ala Ser Thr Met Gln Arg Phe Phe Glu Lys Phe Ala Gly Leu Gly
        355                 360                 365

Ala Arg Thr Gln Thr Phe Met Pro His Phe Gly Leu Ser Glu Thr Gly
    370                 375                 380

Ala Leu Ser Thr Leu Asp Glu Ala Pro Gln Gln Arg Val Leu Glu Leu
385                 390                 395                 400

Asp Ala Asp Ala Leu Asn Lys Arg Lys Arg Val Ala Ala Gly Ala Ser
                405                 410                 415

Gln Ala Arg Val Thr Val Leu Asn Cys Gly Ala Val Asp Gln Asp Val
            420                 425                 430

Glu Leu Arg Ile Val Cys Pro Glu Gly Glu Thr Leu Cys Arg Pro Asp
        435                 440                 445

Glu Ile Gly Glu Ile Trp Val Lys Ser Pro Ala Ile Ala Arg Gly Tyr
    450                 455                 460

Leu Phe Ala Lys Pro Ala Asp Gln Arg Gln Phe Asn Cys Ser Ile Arg
465                 470                 475                 480

His Thr Asp Asp Ser Gly Tyr Phe Arg Thr Gly Asp Leu Gly Phe Ile
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485 490 495

Ala Asp Gly Cys Leu Tyr Val Thr Gly Arg Val Lys Glu Val Leu Ile
500 505 510

Ile Arg Gly Lys Asn His Tyr Pro Ala His Ile Glu Ala Ser Ile Ala
515 520 525

Ala Thr Ala Ser Pro Gly Ala Leu Met Pro Val Val Phe Ser Ile Glu
530 535 540

Arg Gln Asp Glu Glu Arg Val Ala Ala Val Ile Ala Val Asn His Pro
545 550 555 560

Trp Thr Pro Ala Ala Cys Ala Ala Gln Ala His Lys Ile Arg Gln Gln
565 570 575

Val Ala Asp Gln His Gly Val Ala Leu Ala Glu Leu Ala Phe Ala Glu
580 585 590

His Arg His Val Phe Gly Thr Tyr Pro Gly Lys Leu Lys Arg Arg Leu
595 600 605

Val Lys Glu Ala Tyr Val Asn Gly Gln Leu Pro Leu Leu Trp His Glu
610 615 620

Gly Lys Asn Arg Asp Val Pro Ala Ala Ala Ala Asp Asp Arg Gln Ala
625 630 635 640

Gln His Val Ala Asp Leu Cys Arg Lys Val Phe Leu Pro Val Leu Gly
645 650 655

Val Ala Pro Pro His Ala Gln Trp Pro Leu Cys Glu Leu Ala Leu Asp
660 665 670

Ser Leu Gln Cys Val Arg Leu Ala Gly Ala Ile Glu Glu Cys Tyr Gly
675 680 685

Val Pro Phe Glu Pro Thr Leu Leu Phe Lys Leu Glu Thr Val Gly Ala
690 695 700

Ile Ala Glu Tyr Val Leu Ala His Gly Arg Gln Ala Pro Thr Pro Thr
705 710 715 720

Arg Ala Pro Val Ala Ser Thr Thr Cys Ser Glu Glu Pro Ile Ala Ile
725 730 735

Val Ala Met His Cys Glu Val Pro Gly Ala Gly Glu Asn Thr Glu Ala

740 745 750

Leu Trp Ser Phe Leu Arg Ser Asp Val Asn Ala Ile Arg Pro Ile Glu
755 760 765

Ser Thr Arg Pro Asp Leu Trp Ala Ala Met Arg Ala Tyr Pro Gly Leu
770 775 780

Ala Gly Glu Gln Leu Pro Arg Tyr Ala Gly Phe Leu Asp Asp Val Asp
785 790 795 800

Ala Phe Asp Ala Ala Phe Phe Gly Ile Ser Arg Arg Glu Ala Glu Cys
805 810 815

Met Asp Pro Gln Gln Arg Lys Val Leu Glu Met Val Trp Lys Leu Ile
820 825 830

Glu Gln Ala Gly His Asp Pro Leu Ser Trp Gly Gly Gln Pro Val Gly
835 840 845

Leu Phe Val Gly Ala His Thr Ser Asp Tyr Gly Glu Leu Leu Ala Ser
850 355 860

Gln Pro Gln Leu Met Ala Gln Cys Gly Ala Tyr Ile Asp Ser Gly Ser
865 870 875 880

His Leu Thr Met Ile Pro Asn Arg Ala Ser Arg Trp Phe Asn Phe Thr
885 890 895

Gly Pro Ser Glu Val Ile Asn Ser Ala Cys Ser Ser Ser Leu Val Ala
900 905 910

Leu His Arg Ala Val Gln Ser Leu Arg Gln Gly Glu Ser Ser Val Ala
915 920 925

Leu Val Leu Gly Val Asn Leu Ile Leu Ala Pro Lys Val Leu Leu Ala
930 935 940

Ser Ala Ser Ala Gly Met Leu Ser Pro Asp Gly Arg Cys Lys Thr Leu
945 950 955 960

Asp Ala Ala Ala Asp Gly Phe Val Arg Ser Glu Gly Ile Ala Gly Val
965 970 975

Ile Leu Lys Pro Leu Ala Gln Ala Leu Ala Asp Gly Asp Arg Val Tyr
980 985 990

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Gly Leu Val Arg Gly Val Ala Val  Asn His Gly Gly Arg  Ser Asn Ser
        995                 1000                1005

Leu Arg  Ala Pro Asn Val Asn  Ala Gln Arg Gln Leu  Leu Ile Arg
    1010                1015                1020

Thr Tyr  Gln Glu Ala Gly Val  Glu Pro Ala Ser Val  Gly Tyr Val
    1025                1030                1035

Glu Leu  His Gly Thr Gly Thr  Ser Leu Gly Asp Pro  Ile Glu Ile
    1040                1045                1050

Gln Ala  Leu Lys Glu Ala Phe  Ile Ala Leu Gly Ala  Gln Ala Ala
    1055                1060                1065

Pro Ser  Asn Cys Gly Ile Gly  Ser Val Lys Ser Ala  Leu Gly His
    1070                1075                1080

Leu Glu  Ala Ala Ala Gly Leu  Thr Gly Leu Ile Lys  Val Leu Leu
    1085                1090                1095

Met Leu  Lys His Gly Glu Gln  Ala Gly Thr Arg His  Phe Ser Thr
    1100                1105                1110

Leu Asn  Pro Leu Ile Asp Leu  Arg Gly Thr Ser Phe  Glu Val Val
    1115                1120                1125

Ala Gln  His Arg Ala Trp Pro  Ser Gln Val Gly Ile  His Gly Thr
    1130                1135                1140

Leu Leu  Pro Arg Arg Ala Gly  Ile Ser Ser Phe Gly  Phe Gly Gly
    1145                1150                1155

Ala Asn  Ala His Ala Ile Val  Glu Glu His Val Ile  Ala Thr Pro
    1160                1165                1170

Pro Ser  Thr Ser Ser Ala Gly  Gly Pro Val Gly Ile  Val Leu Ser
    1175                1180                1185

Ala Gly  Ser Glu Ala Val Leu  Arg Gln Gln Val Leu  Ala Leu Ser
    1190                1195                1200

Ala Trp  Leu Arg Gln Gln Ser  Pro Thr Pro Ala Gln  Met Ile Asp
    1205                1210                1215

Val Ala  Tyr Thr Leu Gln Val  Gly Arg Ala Ala Leu  Ser His Arg
    1220                1225                1230
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Leu Ala  Phe Ser Ala Thr Asp  Ala Glu Gln Ala Leu  Ala Arg Leu
    1235                 1240                 1245

Glu Gly  Arg Leu Ala Gly Val  Met Asp Ala Glu Val  His His Gly
    1250                 1255                 1260

Val Val  Asp Ala Ala Ala Thr  Ala Pro Glu His Gly  Arg Gln Thr
    1265                 1270                 1275

Arg Glu  Gly Leu Ala Gly Leu  Leu Arg Ala Trp Thr  Gln Gly Val
    1280                 1285                 1290

Arg Val  Asp Trp Ser Ala Leu  Tyr Gly Ile Gln Arg  Pro Gln Arg
    1295                 1300                 1305

Val Ser  Leu Pro Val Tyr Pro  Phe Ala Arg Glu Arg  Tyr Trp Leu
    1310                 1315                 1320

Pro Gly  Gln Ala Met His Ala  Ala Ala Asp Ala His  Pro Met Leu
    1325                 1330                 1335

Gln Leu  Leu His Ala Asn Ala  Lys Leu His Arg Tyr  Ala Leu Arg
    1340                 1345                 1350

Arg Ser  Gly Cys Ala Ser Phe  Leu Val Asp His Cys  Val Asp Gly
    1355                 1360                 1365

Arg Gln  Val Leu Pro Ala Ala  Val Gln Leu Glu Leu  Val Arg Ala
    1370                 1375                 1380

Val Ala  Gln Arg Val Met Ala  Gln Asp Glu Gly Cys  Ile Glu Leu
    1385                 1390                 1395

Ala Gln  Val Ala Phe Leu His  Pro Leu Met Met Glu  Glu Thr Glu
    1400                 1405                 1410

Leu Glu  Val Glu Ile Glu Leu  Ser Lys Ser Asp Gln  Asp Glu Phe
    1415                 1420                 1425

Asp Phe  Gln Leu His Asp Ala  His Arg Gln Gln Val  Phe Ser Gln
    1430                 1435                 1440

Gly His  Val Arg Arg Arg Val  Tyr Thr Ala Thr Pro  Arg Leu Asp
    1445                 1450                 1455

Leu Ala  Gln Leu Gln Lys Leu  Cys Ala Glu Arg Val  Leu Ser Gly
    1460                 1465                 1470
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Glu Asp Cys Tyr Ala His Phe Thr Ala Cys Gly Leu Gln Leu Gly
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Asp Arg Leu Lys Ser Val Gln Ser Ile Gly Cys Gly Arg Asn Gly
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Glu Gly Glu Pro Ile Ala Leu Gly Val Leu Arg Leu Pro Pro Ser
1505 1510 1515

Ser Val Glu Asp Ser His Val Leu Pro Pro Ser Leu Leu Asp Gly
1520 1525 1530

Ala Leu Gln Cys Ser Leu Gly Leu Gln Arg Asp Val Glu His Ile
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Ala Met Pro Tyr Thr Leu Glu Arg Met Thr Val His Ala Pro Ile
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Pro Pro Glu Ala Trp Val Leu Leu Arg His Gly His Ala Ala Arg
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Gln Ser Leu Asp Ile Asp Leu Leu Asp Ser Glu Gly Arg Val Cys
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Val Ser Leu Gly Asn Tyr Thr Gly Arg Ala Pro Lys Ala Val Ser
1595 1600 1605

Ala Val Arg Ala Leu Val Leu Ala Pro Val Trp Gln Ala Leu Thr
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Glu Thr Ala Pro Ala Trp Pro Asp Pro Ala Glu Arg Ile Val Thr
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Val Gly Asp Asp Ala Trp Arg Ser His Phe Gly Phe Asp Glu Pro
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Ala Leu Ser Leu Glu Asp Ser Val Glu Val Ile Ala Thr Arg Leu
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Gly Gln Ser Gly Lys Phe Asp His Leu Val Trp Ile Val Pro Ile
1670 1675 1680

Ala Glu Ser Glu Thr Asp Ile Ala Ala Gln Gly Ser Ala Ala Ile
1685 1690 1695

Ala Gly Phe Arg Leu Val Lys Ala Leu Leu Ala Leu Gly Tyr Ala
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His Arg Pro Leu Gly Leu Thr Val Leu Thr Arg Gln Ala Leu Thr
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Arg Gln Pro Ser His Ala Ala Val His Gly Leu Ile Gly Thr Leu
1730 1735 1740

Ala Lys Glu Tyr Cys Asn Trp Lys Ile Arg Leu Leu Asp Leu Pro
1745 1750 1755

Ser Val Lys Ser Trp Pro Gln Trp Glu Gln Leu Arg Ser Leu Pro
1760 1765 1770

Trp His Ala Gln Gly Glu Ala Leu Ile Gly Arg Gly Thr Cys Trp
1775 1780 1785

Tyr Arg Arg Gln Leu Cys Glu Val Leu Pro Leu Pro Ser Leu Glu
1790 1795 1800

Pro Pro Pro Tyr Arg Val Gly Gly Val Tyr Val Val Ile Gly Gly
1805 1810 1815

Ala Gly Gly Leu Gly Glu Val Leu Ser Glu His Leu Ile Arg Thr
1820 1825 1830

Tyr Asp Ala Gln Leu Ile Trp Ile Gly Arg Arg Val Leu Asp Glu
1835 1840 1845

Gly Ile Ala Arg Lys Gln Thr Arg Leu Ala Ser Leu Gly Arg Ala
1850 1855 1860

Pro His Tyr Ile Ser Ala Asp Ala Ser Asp Pro Ala Ala Leu Gln
1865 1870 1875

Ala Ala His Asn Glu Ile Val Ala Leu His Gly Gln Pro His Gly
1880 1885 1890

Leu Ile Leu Ser Asn Ile Val Leu Lys Asp Ala Ser Leu Ala Arg
1895 1900 1905

Met Glu Glu Ala Asp Phe Arg Asp Val Leu Ala Ala Lys Leu Asp
1910 1915 1920

Val Ser Val Cys Ala Ala Gln Val Phe Gly Thr Ala Pro Leu Asp
1925 1930 1935

Phe Val Leu Phe Phe Ser Ser Ile Gln Ser Thr Thr Lys Ala Ala

1940 1945 1950

Gly Gln Gly Asn Tyr Ala Ala Gly Cys Cys Tyr Val Asp Ala Phe
1955 1960 1965

Gly Glu Leu Trp Ala Arg Arg Gly Leu Arg Val Lys Thr Ile Asn
1970 1975 1980

Trp Gly Tyr Trp Gly Ser Val Gly Val Val Ala Gly Glu Asp Tyr
1985 1990 1995

Arg Arg Arg Met Ala Gln Lys His Met Ala Ser Ile Glu Gly Ala
2000 2005 2010

Glu Ala Met Gln Val Leu Ser Gln Leu Leu Cys Ala Pro Leu Gln
2015 2020 2025

Arg Leu Ala Tyr Val Lys Ile Asp Asp Ala Asn Ala Met Arg Ala
2030 2035 2040

Leu Gly Val Val Glu Asp Glu Ser Val Gln Ile Pro Val His Ala
2045 2050 2055

Pro Ala Glu Pro Pro Arg Gly Gln Pro Gly Pro Val Val Glu Leu
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Ser Val Asn Leu Asp Ala Arg Arg Glu Arg Glu Thr Leu Leu Ala
2075 2080 2085

Ala Trp Leu Leu Glu Leu Ile Glu Gln Leu Gly Gly Phe Pro Pro
2090 2095 2100

Ala Ser Phe Asp Ile Ala Thr Leu Ala Gln Arg Leu His Ile Val
2105 2110 2115

Pro Ala Tyr Arg Ser Trp Leu Glu His Ser Val Arg Met Leu Gly
2120 2125 2130

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        275                 280                 285

agt ttc atc cat ccg cac agt tat atg aaa cga ccc ggc ttc tgg ctg      912
Ser Phe Ile His Pro His Ser Tyr Met Lys Arg Pro Gly Phe Trp Leu
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290                 295                 300

gag acg gtt gcg gct aga gac gcc acg cac atg gcc gcg ccg aac ttc      960
Glu Thr Val Ala Ala Arg Asp Ala Thr His Met Ala Ala Pro Asn Phe
305                 310                 315                 320

gcg ttc gac tac tgc tgc gac tgg gtg atg gtc gag cag ctt ccg ccg     1008
Ala Phe Asp Tyr Cys Cys Asp Trp Val Met Val Glu Gln Leu Pro Pro
                325                 330                 335

tct gcg ttg tct acg ctt acg cat atc gtg tgt ggc ggc gag ccg gtg     1056
Ser Ala Leu Ser Thr Leu Thr His Ile Val Cys Gly Gly Glu Pro Val
            340                 345                 350

cgc gcc tcg acc atg cag cgc ttc ttc gag aaa ttc gcc gga ctc ggt     1104
Arg Ala Ser Thr Met Gln Arg Phe Phe Glu Lys Phe Ala Gly Leu Gly
        355                 360                 365

gcg cgt acg cag act ttc atg ccg cac ttc ggc ttg tct gaa acc ggt     1152
Ala Arg Thr Gln Thr Phe Met Pro His Phe Gly Leu Ser Glu Thr Gly
    370                 375                 380

gcg ctg agt acc ttg gac gag gcg ccc caa cag cgc gtc ttg gaa cta     1200
Ala Leu Ser Thr Leu Asp Glu Ala Pro Gln Gln Arg Val Leu Glu Leu
385                 390                 395                 400

gat gcc gac gcc ttg aac aaa cgc aag cgc gtg gcg gca ggg gcg agc     1248
Asp Ala Asp Ala Leu Asn Lys Arg Lys Arg Val Ala Ala Gly Ala Ser
                405                 410                 415

cag gcg cgt gtg aca gtg ctc aat tgc ggc gcc gtc gac caa gat gtg     1296
Gln Ala Arg Val Thr Val Leu Asn Cys Gly Ala Val Asp Gln Asp Val
            420                 425                 430

gag ttg cgt atc gtc tgt cct gaa ggc gag acg ttg tgc aga cca gat     1344
Glu Leu Arg Ile Val Cys Pro Glu Gly Glu Thr Leu Cys Arg Pro Asp
        435                 440                 445

gag atc ggc gaa ata tgg gta aag tcg cct gcg atc gcc cgt ggc tac     1392
Glu Ile Gly Glu Ile Trp Val Lys Ser Pro Ala Ile Ala Arg Gly Tyr
    450                 455                 460

ctg ttt gcg aag ccc gcc gat cag cga cag ttc aac tgc agc atc cgt     1440
Leu Phe Ala Lys Pro Ala Asp Gln Arg Gln Phe Asn Cys Ser Ile Arg
465                 470                 475                 480

cat acc gac gat agc ggt tac ttt cgt acc ggc gac ctg ggt ttc att     1488
His Thr Asp Asp Ser Gly Tyr Phe Arg Thr Gly Asp Leu Gly Phe Ile
                485                 490                 495

gcc gat ggc tgt ctg tat gtc acc gga agg gta aag gag gtg ctg atc     1536
Ala Asp Gly Cys Leu Tyr Val Thr Gly Arg Val Lys Glu Val Leu Ile
            500                 505                 510

ata cgc ggt aag aat cat tac ccc gca cat atc gaa gcc tcg atc gcc     1584
Ile Arg Gly Lys Asn His Tyr Pro Ala His Ile Glu Ala Ser Ile Ala
        515                 520                 525

gct acc gca tcg cct ggc gcg ctg atg ccg gtg gtg ttc agc atc gag     1632
Ala Thr Ala Ser Pro Gly Ala Leu Met Pro Val Val Phe Ser Ile Glu
    530                 535                 540

cgg cag gac gag gag cgc gta gct gcg gtg atc gcc gtc aat cac ccg     1680
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Arg Gln Asp Glu Glu Arg Val Ala Ala Val Ile Ala Val Asn His Pro
545                 550                 555                 560

tgg acg ccg gca gca tgc gcc gcg cag gca cac aag atc cgg caa cag   1728
Trp Thr Pro Ala Ala Cys Ala Ala Gln Ala His Lys Ile Arg Gln Gln
                565                 570                 575

gta gcc gac cag cat gga gtc gcc ctg gcg gag cta gcc ttt gcc gaa   1776
Val Ala Asp Gln His Gly Val Ala Leu Ala Glu Leu Ala Phe Ala Glu
            580                 585                 590

cac cgg cac gtg ttc ggc acc tat ccg ggc aaa ctg aag cgg cgc cta   1824
His Arg His Val Phe Gly Thr Tyr Pro Gly Lys Leu Lys Arg Arg Leu
        595                 600                 605

gtc aag gaa gcc tat gtc aac ggc cag ctg ccg ttg tta tgg cat gag   1872
Val Lys Glu Ala Tyr Val Asn Gly Gln Leu Pro Leu Leu Trp His Glu
    610                 615                 620

ggt aag aac cgg gac gta cca gcg gcc gcc gcg gac gat cgg cag gcg   1920
Gly Lys Asn Arg Asp Val Pro Ala Ala Ala Ala Asp Asp Arg Gln Ala
625                 630                 635                 640

caa cac gtg gcg gac ctg tgt cgg aag gtc ttt ttg ccg gtg ttg ggt   1968
Gln His Val Ala Asp Leu Cys Arg Lys Val Phe Leu Pro Val Leu Gly
                645                 650                 655

gtc gcg ccg ccg cat gcc caa tgg ccg ctg tgc gaa ctg gcg ctg gat   2016
Val Ala Pro Pro His Ala Gln Trp Pro Leu Cys Glu Leu Ala Leu Asp
            660                 665                 670
tcg ctc caa tgc gtg cgt ctt gcc ggt gcc atc gaa gag tgc tac ggc   2064
Ser Leu Gln Cys Val Arg Leu Ala Gly Ala Ile Glu Glu Cys Tyr Gly
        675                 680                 685

gtg cct ttc gaa ccc acg ttg cta ttc aag ctt gag acg gtc ggg gca   2112
Val Pro Phe Glu Pro Thr Leu Leu Phe Lys Leu Glu Thr Val Gly Ala
    690                 695                 700

atc gcc gaa tat gtc ctg gcg cac gga cgt cag gcg ccc acg ccg acg   2160
Ile Ala Glu Tyr Val Leu Ala His Gly Arg Gln Ala Pro Thr Pro Thr
705                 710                 715                 720

cgt gcg ccg gtg gca agc aca aca tgc tca gag gaa ccg atc gcc att   2208
Arg Ala Pro Val Ala Ser Thr Thr Cys Ser Glu Glu Pro Ile Ala Ile
                725                 730                 735

gtg gcg atg cac tgt gag gtg ccc gga gcg ggc gag aac act gaa gca   2256
Val Ala Met His Cys Glu Val Pro Gly Ala Gly Glu Asn Thr Glu Ala
            740                 745                 750

ttg tgg tcg ttc ctg cgg agc gac gtc aac gcg atc cgg ccg atc gaa   2304
Leu Trp Ser Phe Leu Arg Ser Asp Val Asn Ala Ile Arg Pro Ile Glu
        755                 760                 765

tca acg cgc ccg gac tta tgg gca gcg atg cgc gcc tat ccc ggc ctc   2352
Ser Thr Arg Pro Asp Leu Trp Ala Ala Met Arg Ala Tyr Pro Gly Leu
    770                 775                 780

gcg ggc gaa cag ctg ccg cgc tat gcg ggt ttc ctc gac gac gtt gat   2400
Ala Gly Glu Gln Leu Pro Arg Tyr Ala Gly Phe Leu Asp Asp Val Asp
785                 790                 795                 800

gct ttc gat gct gcg ttt ttc ggt atc tcg cgt cgc gag gcc gaa tgc   2448
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Ala Phe Asp Ala Ala Phe Phe Gly Ile Ser Arg Arg Glu Ala Glu Cys
805                 810                 815

atg gac ccg cag cag cgc aaa gtg ctg gag atg gtg tgg aag ctg atc      2496
Met Asp Pro Gln Gln Arg Lys Val Leu Glu Met Val Trp Lys Leu Ile
820                 825                 830

gag caa gcc ggt cac gat ccg ctg tcc tgg ggc ggc cag ccg gtc ggc      2544
Glu Gln Ala Gly His Asp Pro Leu Ser Trp Gly Gly Gln Pro Val Gly
835                 840                 845

ctg ttc gtg ggt gcg cat acg tcc gac tat ggc gag ctg ctg gcg agc      2592
Leu Phe Val Gly Ala His Thr Ser Asp Tyr Gly Glu Leu Leu Ala Ser
850                 855                 860

cag ccg caa ctg atg gcc caa tgt ggc gct tac atc gat tcg ggt tcg      2640
Gln Pro Gln Leu Met Ala Gln Cys Gly Ala Tyr Ile Asp Ser Gly Ser
865                 870                 875                 880

cat ttg acc atg att ccg aac cgg gct tcg cgc tgg ttc aat ttc acc      2688
His Leu Thr Met Ile Pro Asn Arg Ala Ser Arg Trp Phe Asn Phe Thr
885                 890                 895

ggc ccc agc gaa gta atc aac agc gct tgc tcc agc tcg ctg gtg gcg      2736
Gly Pro Ser Glu Val Ile Asn Ser Ala Cys Ser Ser Ser Leu Val Ala
900                 905                 910

ctg cat cgg gcg gtt caa tcg ctg cgc caa ggc gaa agc agt gtc gcc      2784
Leu His Arg Ala Val Gln Ser Leu Arg Gln Gly Glu Ser Ser Val Ala
915                 920                 925

ctg gta ctc ggc gtg aac ctt atc ctg gct ccc aag gtg ctg tta gcc      2832
Leu Val Leu Gly Val Asn Leu Ile Leu Ala Pro Lys Val Leu Leu Ala
930                 935                 940

agt gca agc gcg ggc atg ctt tcg ccc gat ggc cgc tgc aag acg ctt      2880
Ser Ala Ser Ala Gly Met Leu Ser Pro Asp Gly Arg Cys Lys Thr Leu
945                 950                 955                 960

gac gcc gcc gcc gat ggc ttc gtg cgt tcg gaa ggg atc gca ggg gtg      2928
Asp Ala Ala Ala Asp Gly Phe Val Arg Ser Glu Gly Ile Ala Gly Val
965                 970                 975

ata ttg aag cca ctg gcg cag gcg ctg gcc gat ggt gac agg gtc tac      2976
Ile Leu Lys Pro Leu Ala Gln Ala Leu Ala Asp Gly Asp Arg Val Tyr
980                 985                 990

ggt cta gtc cgc ggc gtg gcg gtc  aac cat ggc ggc cgt  tcc aat tcc    3024
Gly Leu Val Arg Gly Val Ala Val  Asn His Gly Gly Arg  Ser Asn Ser
995                 1000                1005

ttg cgt  gct ccc aac gtc aac  gcg cag cgg caa ctg  ctg atc cgg       3069
Leu Arg  Ala Pro Asn Val Asn  Ala Gln Arg Gln Leu  Leu Ile Arg
1010                1015                1020

act tac  cag gaa gcc ggt gtc  gag ccg gcc agc gtc  ggt tat gtt       3114
Thr Tyr  Gln Glu Ala Gly Val  Glu Pro Ala Ser Val  Gly Tyr Val
1025                1030                1035

gaa cta  cac ggc act ggt acc  agc ctg ggt gat ccg  atc gaa atc       3159
Glu Leu  His Gly Thr Gly Thr  Ser Leu Gly Asp Pro  Ile Glu Ile
1040                1045                1050
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cag gcg  ctg aag gaa gct ttc  att gcg ttg ggg gca  cag gcc gcc      3204
Gln Ala  Leu Lys Glu Ala Phe  Ile Ala Leu Gly Ala  Gln Ala Ala
    1055                 1060                 1065

ccg tca  aac tgc ggc atc ggt  tcg gtg aag tcc gcg  ctg ggc cat      3249
Pro Ser  Asn Cys Gly Ile Gly  Ser Val Lys Ser Ala  Leu Gly His
    1070                 1075                 1080

cta gaa  gcc gct gca ggc ctg  acc ggc ctg atc aag  gtg ctg ctg      3294
Leu Glu  Ala Ala Ala Gly Leu  Thr Gly Leu Ile Lys  Val Leu Leu
    1085                 1090                 1095

atg ctc  aag cac ggc gag cag  gcc ggc acg cgc cat  ttc agc acg      3339
Met Leu  Lys His Gly Glu Gln  Ala Gly Thr Arg His  Phe Ser Thr
    1100                 1105                 1110

ctc aat  ccg ctg atc gat ttg  cga ggt acg tca ttc  gaa gtg gtg      3384
Leu Asn  Pro Leu Ile Asp Leu  Arg Gly Thr Ser Phe  Glu Val Val
    1115                 1120                 1125

gcg cag  cat cgc gca tgg ccg  tcg cag gtc ggc att  cac ggc aca      3429
Ala Gln  His Arg Ala Trp Pro  Ser Gln Val Gly Ile  His Gly Thr
    1130                 1135                 1140

ctc ttg  ccg cgt cgc gcg ggt  atc agc tca ttc ggc  ttc ggc ggc      3474
Leu Leu  Pro Arg Arg Ala Gly  Ile Ser Ser Phe Gly  Phe Gly Gly
    1145                 1150                 1155

gcc aat  gcg cat gcg atc gtg  gaa gag cat gtc att  gcc acg ccc      3519
Ala Asn  Ala His Ala Ile Val  Glu Glu His Val Ile  Ala Thr Pro
    1160                 1165                 1170

ccc tcg  acg agc tcc gct ggc  ggc ccg gta ggt atc  gtg ttg tca      3564
Pro Ser  Thr Ser Ser Ala Gly  Gly Pro Val Gly Ile  Val Leu Ser
    1175                 1180                 1185

gcc ggt  agt gaa gct gtc ttg  cgg caa caa gtg ctg  gcc ttg tca      3609
Ala Gly  Ser Glu Ala Val Leu  Arg Gln Gln Val Leu  Ala Leu Ser
    1190                 1195                 1200

gcc tgg  cta agg cag caa tcg  ccg aca ccc gcg caa  atg atc gat      3654
Ala Trp  Leu Arg Gln Gln Ser  Pro Thr Pro Ala Gln  Met Ile Asp
    1205                 1210                 1215

gtc gcc  tac acc tta cag gta  gga cgc gca gcc ctg  tcg cac agg      3699
Val Ala  Tyr Thr Leu Gln Val  Gly Arg Ala Ala Leu  Ser His Arg
    1220                 1225                 1230

ttg gct  ttt agc gcg acg gac  gcc gag cag gca ttg  gcg agg ctt      3744
Leu Ala  Phe Ser Ala Thr Asp  Ala Glu Gln Ala Leu  Ala Arg Leu
    1235                 1240                 1245

gag ggt  cgt ctg gcg ggc gtg  atg gat gcc gag gtc  cat cac ggt      3789
Glu Gly  Arg Leu Ala Gly Val  Met Asp Ala Glu Val  His His Gly
    1250                 1255                 1260

gtc gtg  gat gct gcc gca acg  gct ccc gaa cat ggg  cgg cag acg      3834
Val Val  Asp Ala Ala Ala Thr  Ala Pro Glu His Gly  Arg Gln Thr
    1265                 1270                 1275

cgc gaa  ggt ctt gcc ggt ttg  ctg cga gcc tgg act  cag ggc gtg      3879
Arg Glu  Gly Leu Ala Gly Leu  Leu Arg Ala Trp Thr  Gln Gly Val
    1280                 1285                 1290
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cgc gtc  gat tgg tcg gcg ctg  tac ggc ata cag cga  ccg cag cgc      3924
Arg Val  Asp Trp Ser Ala Leu  Tyr Gly Ile Gln Arg  Pro Gln Arg
    1295                 1300                 1305

gtt agc  ctg cct gtc tac ccc  ttc gct agg gaa cgc  tat tgg ctg      3969
Val Ser  Leu Pro Val Tyr Pro  Phe Ala Arg Glu Arg  Tyr Trp Leu
    1310                 1315                 1320

ccc ggc  cag gct atg cat gcc  gct gcg gac gct cat  ccg atg ctg      4014
Pro Gly  Gln Ala Met His Ala  Ala Ala Asp Ala His  Pro Met Leu
    1325                 1330                 1335

cag ctg  ttg cat gcc aat gcc  aaa cta cat cgc tac  gcc ttg cgt      4059
Gln Leu  Leu His Ala Asn Ala  Lys Leu His Arg Tyr  Ala Leu Arg
    1340                 1345                 1350

agg tcc  ggc tgc gca agc ttt  ctt gtt gat cat tgc  gtg gat ggt      4104
Arg Ser  Gly Cys Ala Ser Phe  Leu Val Asp His Cys  Val Asp Gly
    1355                 1360                 1365

cga cag  gta cta ccg gca gcc  gtg caa ctg gaa ttg  gtg cgc gcc      4149
Arg Gln  Val Leu Pro Ala Ala  Val Gln Leu Glu Leu  Val Arg Ala
    1370                 1375                 1380
gtg gcg  cag cgg gtc atg gcg  cag gat gag ggt tgt  atc gaa ctg      4194
Val Ala  Gln Arg Val Met Ala  Gln Asp Glu Gly Cys  Ile Glu Leu
    1385                 1390                 1395

gcg cag  gtc gcc ttt ttg cat  ccc ctc atg atg gag  gag act gag      4239
Ala Gln  Val Ala Phe Leu His  Pro Leu Met Met Glu  Glu Thr Glu
    1400                 1405                 1410

ctg gag  gtc gaa atc gaa ctg  tcg aag agc gat caa  gat gag ttc      4284
Leu Glu  Val Glu Ile Glu Leu  Ser Lys Ser Asp Gln  Asp Glu Phe
    1415                 1420                 1425

gat ttc  caa ctt cac gat gct  cac cgc caa cag gtc  ttt agc cag      4329
Asp Phe  Gln Leu His Asp Ala  His Arg Gln Gln Val  Phe Ser Gln
    1430                 1435                 1440

ggg cac  gta cgt cgc cgg gtc  tat acg gcg aca ccg  cgc ttg gat      4374
Gly His  Val Arg Arg Arg Val  Tyr Thr Ala Thr Pro  Arg Leu Asp
    1445                 1450                 1455

tta gcc  cag ctg caa aag ctt  tgt gcc gag cgc gtg  ttg tcc ggc      4419
Leu Ala  Gln Leu Gln Lys Leu  Cys Ala Glu Arg Val  Leu Ser Gly
    1460                 1465                 1470

gaa gac  tgt tat gcg cac ttc  acc gcc tgc gga ttg  cag ctc ggc      4464
Glu Asp  Cys Tyr Ala His Phe  Thr Ala Cys Gly Leu  Gln Leu Gly
    1475                 1480                 1485

gac cgg  ctc aaa tcc gtg caa  tcg atc ggc tgc gga  cgc aat ggc      4509
Asp Arg  Leu Lys Ser Val Gln  Ser Ile Gly Cys Gly  Arg Asn Gly
    1490                 1495                 1500

gag ggc  gag ccg atc gca ttg  ggt gtc ctg cgc ctg  cca cca tca      4554
Glu Gly  Glu Pro Ile Ala Leu  Gly Val Leu Arg Leu  Pro Pro Ser
    1505                 1510                 1515

agc gtt  gaa gac agc cat gtg  ctg cct cct agc ctg  ctt gat ggt      4599
Ser Val  Glu Asp Ser His Val  Leu Pro Pro Ser Leu  Leu Asp Gly
    1520                 1525                 1530
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gcc ttg  cag tgt agc ctt ggc  ttg cag cgt gat gtc  gag cac atc      4644
Ala Leu  Gln Cys Ser Leu Gly  Leu Gln Arg Asp Val  Glu His Ile
    1535                 1540                 1545

gcc atg  cca tac acg ctg gag  cgg atg acg gtg cat  gcg ccg att      4689
Ala Met  Pro Tyr Thr Leu Glu  Arg Met Thr Val His  Ala Pro Ile
    1550                 1555                 1560

cct ccc  gag gcc tgg gtg ctg  ctg cgt cac ggc cat  gca gcc aga      4734
Pro Pro  Glu Ala Trp Val Leu  Leu Arg His Gly His  Ala Ala Arg
    1565                 1570                 1575

cag tcc  ctg gac atc gat ctc  ctg gat tcc gaa ggt  agg gtc tgc      4779
Gln Ser  Leu Asp Ile Asp Leu  Leu Asp Ser Glu Gly  Arg Val Cys
    1580                 1585                 1590

gtc agc  ctc ggc aat tac acc  ggc cgt gca ccg aaa  gcc gtt tcc      4824
Val Ser  Leu Gly Asn Tyr Thr  Gly Arg Ala Pro Lys  Ala Val Ser
    1595                 1600                 1605

gcc gtc  agg gcg ctt gtc ttg  gca ccg gtc tgg caa  gcg ttg acc      4869
Ala Val  Arg Ala Leu Val Leu  Ala Pro Val Trp Gln  Ala Leu Thr
    1610                 1615                 1620

gaa acg  gcg ccg gca tgg ccc  gat ccg gcc gaa cgc  atc gtt acg      4914
Glu Thr  Ala Pro Ala Trp Pro  Asp Pro Ala Glu Arg  Ile Val Thr
    1625                 1630                 1635

gta gga  gac gat gca tgg cgt  agt cac ttc ggt ttc  gac gag ccg      4959
Val Gly  Asp Asp Ala Trp Arg  Ser His Phe Gly Phe  Asp Glu Pro
    1640                 1645                 1650

gcc ttg  tcc ctg gag gac agc  gtc gaa gtc atc gcg  acg cga ctg      5004
Ala Leu  Ser Leu Glu Asp Ser  Val Glu Val Ile Ala  Thr Arg Leu
    1655                 1660                 1665

ggc cag  agc ggc aag ttc gat  cat cta gtc tgg atc  gtg ccg ata      5049
Gly Gln  Ser Gly Lys Phe Asp  His Leu Val Trp Ile  Val Pro Ile
    1670                 1675                 1680

gcc gag  agt gaa acc gat att  gca gcg caa ggt tca  gcg gcg atc      5094
Ala Glu  Ser Glu Thr Asp Ile  Ala Ala Gln Gly Ser  Ala Ala Ile
    1685                 1690                 1695

gcc ggt  ttc cgg ttg gtc aag  gcg ttg ctt gcg ttg  ggc tat gcg      5139
Ala Gly  Phe Arg Leu Val Lys  Ala Leu Leu Ala Leu  Gly Tyr Ala
    1700                 1705                 1710

cat cgc  ccg ctg ggt ctc acc  gtg ctg act cgc caa  gcc ctt acg      5184
His Arg  Pro Leu Gly Leu Thr  Val Leu Thr Arg Gln  Ala Leu Thr
    1715                 1720                 1725

cgg cag  ccg tcg cac gcg gca  gtg cac ggg ctg atc  ggg acg ctg      5229
Arg Gln  Pro Ser His Ala Ala  Val His Gly Leu Ile  Gly Thr Leu
    1730                 1735                 1740

gcc aag  gaa tac tgc aac tgg  aaa atc cgt ctg ctc  gac ctg ccg      5274
Ala Lys  Glu Tyr Cys Asn Trp  Lys Ile Arg Leu Leu  Asp Leu Pro
    1745                 1750                 1755

agc gta  aaa tct tgg ccg caa  tgg gag caa ttg cgg  tcg ttg cct      5319
Ser Val  Lys Ser Trp Pro Gln  Trp Glu Gln Leu Arg  Ser Leu Pro
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    1760                1765                1770

tgg cat  gcg cag ggc gaa gcc  ctg atc ggc cgt ggg  act tgt tgg      5364
Trp His  Ala Gln Gly Glu Ala  Leu Ile Gly Arg Gly  Thr Cys Trp
    1775                1780                1785

tat cgg  cgg cag ttg tgt gaa  gtg ctg ccg ctg ccg  tcg ttg gaa      5409
Tyr Arg  Arg Gln Leu Cys Glu  Val Leu Pro Leu Pro  Ser Leu Glu
    1790                1795                1800

ccg ccg  ccg tac cgc gta ggc  ggt gtc tac gtc gtg  atc ggc ggc      5454
Pro Pro  Pro Tyr Arg Val Gly  Gly Val Tyr Val Val  Ile Gly Gly
    1805                1810                1815

gct ggc  ggc ttg ggt gaa gta  ttg agc gaa cac ttg  atc cgc acg      5499
Ala Gly  Gly Leu Gly Glu Val  Leu Ser Glu His Leu  Ile Arg Thr
    1820                1825                1830

tac gac  gcg cag ctg atc tgg  atc ggg cgg cgc gtg  ctg gac gaa      5544
Tyr Asp  Ala Gln Leu Ile Trp  Ile Gly Arg Arg Val  Leu Asp Glu
    1835                1840                1845

ggc att  gcg cgc aag cag acc  cgg ctt gcg tcg ctg  ggc cgc gca      5589
Gly Ile  Ala Arg Lys Gln Thr  Arg Leu Ala Ser Leu  Gly Arg Ala
    1850                1855                1860

ccg cat  tac atc tcc gcg gac  gcg agt gac ccg gct  gcc ctg cag      5634
Pro His  Tyr Ile Ser Ala Asp  Ala Ser Asp Pro Ala  Ala Leu Gln
    1865                1870                1875

gcg gca  cat aat gag atc gtt  gcg ctg cat ggc cag  ccc cat ggg      5679
Ala Ala  His Asn Glu Ile Val  Ala Leu His Gly Gln  Pro His Gly
    1880                1885                1890

ctc atc  cta agc aac atc gtg  ctg aag gat gcc agt  ctg gct cgt      5724
Leu Ile  Leu Ser Asn Ile Val  Leu Lys Asp Ala Ser  Leu Ala Arg
    1895                1900                1905

atg gag  gaa gcc gat ttc cgt  gac gtg ctg gcc gcg  aaa ctc gac      5769
Met Glu  Glu Ala Asp Phe Arg  Asp Val Leu Ala Ala  Lys Leu Asp
    1910                1915                1920

gtc agc  gtg tgt gcg gca cag  gtg ttc ggc acg gcc  ccc ctt gat      5814
Val Ser  Val Cys Ala Ala Gln  Val Phe Gly Thr Ala  Pro Leu Asp
    1925                1930                1935

ttc gtg  ctg ttt ttt tct tcc  atc cag agc act acc  aag gcg gcc      5859
Phe Val  Leu Phe Phe Ser Ser  Ile Gln Ser Thr Thr  Lys Ala Ala
    1940                1945                1950

ggg caa  ggt aac tac gcc gcc  ggc tgc tgc tat gtc  gac gct ttc      5904
Gly Gln  Gly Asn Tyr Ala Ala  Gly Cys Cys Tyr Val  Asp Ala Phe
    1955                1960                1965

ggc gag  cta tgg gcg cgc cgg  ggt ttg agg gta aag  acc atc aac      5949
Gly Glu  Leu Trp Ala Arg Arg  Gly Leu Arg Val Lys  Thr Ile Asn
    1970                1975                1980

tgg ggc  tac tgg ggc agc gtg  ggc gtc gta gcg ggc  gag gac tat      5994
Trp Gly  Tyr Trp Gly Ser Val  Gly Val Val Ala Gly  Glu Asp Tyr
    1985                1990                1995

cgc cgg  cgc atg gcg caa aaa  cac atg gct tcg att  gag ggt gcc      6039
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Arg Arg  Arg Met Ala Gln Lys  His Met Ala Ser Ile  Glu Gly Ala
    2000                 2005                 2010

gaa gcg  atg cag gtg ttg tcg  cag ttg ttg tgt gcg  ccg ttg caa      6084
Glu Ala  Met Gln Val Leu Ser  Gln Leu Leu Cys Ala  Pro Leu Gln
    2015                 2020                 2025

cgg ctt  gcc tac gtc aag atc  gac gat gct aac gca  atg cgc gct      6129
Arg Leu  Ala Tyr Val Lys Ile  Asp Asp Ala Asn Ala  Met Arg Ala
    2030                 2035                 2040

ctg ggc  gta gta gag gac gag  agc gtg caa atc cct  gtg cac gca      6174
Leu Gly  Val Val Glu Asp Glu  Ser Val Gln Ile Pro  Val His Ala
    2045                 2050                 2055

ccg gcc  gag cct ccc aga ggg  cag cct ggt ccc gtg  gtc gag ttg      6219
Pro Ala  Glu Pro Pro Arg Gly  Gln Pro Gly Pro Val  Val Glu Leu
    2060                 2065                 2070
tcg gtg  aat ctg gat gcc cgg  cgc gaa cgg gaa act  ttg ctg gcg      6264
Ser Val  Asn Leu Asp Ala Arg  Arg Glu Arg Glu Thr  Leu Leu Ala
    2075                 2080                 2085

gcc tgg  ctg ctt gag ttg atc  gag caa ctc ggt ggt  ttt ccg ccg      6309
Ala Trp  Leu Leu Glu Leu Ile  Glu Gln Leu Gly Gly  Phe Pro Pro
    2090                 2095                 2100

gca agt  ttc gac atc gct acg  ctt gcg caa cgc ctg  cac atc gta      6354
Ala Ser  Phe Asp Ile Ala Thr  Leu Ala Gln Arg Leu  His Ile Val
    2105                 2110                 2115

ccc gcc  tat cga agc tgg ctg  gaa cac agc gtg cgg  atg ctc ggc      6399
Pro Ala  Tyr Arg Ser Trp Leu  Glu His Ser Val Arg  Met Leu Gly
    2120                 2125                 2130

gtg tat  ggt tac ctc aga gcg  acg ggg gaa agc cga  ttc gag ctg      6444
Val Tyr  Gly Tyr Leu Arg Ala  Thr Gly Glu Ser Arg  Phe Glu Leu
    2135                 2140                 2145

gcc gac  aag ccg ccc gat gat  gcc agg ggt gcc tgg  aac gcg cat      6489
Ala Asp  Lys Pro Pro Asp Asp  Ala Arg Gly Ala Trp  Asn Ala His
    2150                 2155                 2160

gtg cac  gag gcc agc gtc gaa  gcc ggt gaa gag gca  cag cgg cgt      6534
Val His  Glu Ala Ser Val Glu  Ala Gly Glu Glu Ala  Gln Arg Arg
    2165                 2170                 2175

ctg ctc  gat cgc tgc atg cgg  gcg ttg ccg gcg gtc  ctt cga ggc      6579
Leu Leu  Asp Arg Cys Met Arg  Ala Leu Pro Ala Val  Leu Arg Gly
    2180                 2185                 2190

gaa cgc  aag gcc acc gaa ttg  ctg ttt ccg gaa ggt  tcg atg gcg      6624
Glu Arg  Lys Ala Thr Glu Leu  Leu Phe Pro Glu Gly  Ser Met Ala
    2195                 2200                 2205

tgg gtc  gag ggt atc tac cag  aac aac ccg ctt gcc  gat tac ttc      6669
Trp Val  Glu Gly Ile Tyr Gln  Asn Asn Pro Leu Ala  Asp Tyr Phe
    2210                 2215                 2220

aac gca  caa cta gtc acg cga  ctg att gcc tac ttg  aga cga cga      6714
Asn Ala  Gln Leu Val Thr Arg  Leu Ile Ala Tyr Leu  Arg Arg Arg
    2225                 2230                 2235

cta gag  tcg acg cct acg gcg  cgc ctg aag ctg tgc  gag atc ggc      6759
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Leu Glu  Ser Thr Pro Thr Ala  Arg Leu Lys Leu Cys  Glu Ile Gly
    2240                 2245                 2250

gcc ggc  agc ggt ggt act act  gca agc gtg cta caa  cag ttg cag      6804
Ala Gly  Ser Gly Gly Thr Thr  Ala Ser Val Leu Gln  Gln Leu Gln
    2255                 2260                 2265

gca tat  ggt gag cat att gag  gaa tat ctc tat acc  gac ctg tcg      6849
Ala Tyr  Gly Glu His Ile Glu  Glu Tyr Leu Tyr Thr  Asp Leu Ser
    2270                 2275                 2280

cct gtc  ttc ctg cat cat gcg  gaa aaa cac tat cag  cca cga gcg      6894
Pro Val  Phe Leu His His Ala  Glu Lys His Tyr Gln  Pro Arg Ala
    2285                 2290                 2295

cct tat  ttg agg acc gcc tgt  ttc gac gta gcg cgc  gcg ccg acg      6939
Pro Tyr  Leu Arg Thr Ala Cys  Phe Asp Val Ala Arg  Ala Pro Thr
    2300                 2305                 2310

gcg cag  gcc ctg gaa tct ggc  ggc tac gac gtg gtg  att gcc gcc      6984
Ala Gln  Ala Leu Glu Ser Gly  Gly Tyr Asp Val Val  Ile Ala Ala
    2315                 2320                 2325

aac gta  ctg cat gct acg cgc  gat atc gcc aag acc  ttg cgc aat      7029
Asn Val  Leu His Ala Thr Arg  Asp Ile Ala Lys Thr  Leu Arg Asn
    2330                 2335                 2340

gcg aag  gca ctc ctc aaa cct  ggc ggt ctg ctc ttg  ctc aac gaa      7074
Ala Lys  Ala Leu Leu Lys Pro  Gly Gly Leu Leu Leu  Leu Asn Glu
    2345                 2350                 2355

gtg atc  gag cgc agc ctc gtc  ttg cac ctg act ttc  ggt ctg ctg      7119
Val Ile  Glu Arg Ser Leu Val  Leu His Leu Thr Phe  Gly Leu Leu
    2360                 2365                 2370

gag agc  tgg tgg ttg ccc cag  gac aag atc ttg cgc  ctt gcc ggc      7164
Glu Ser  Trp Trp Leu Pro Gln  Asp Lys Ile Leu Arg  Leu Ala Gly
    2375                 2380                 2385

tcg ccg  ttg ctg gct tgc gcc  acc tgg cgc agc ctg  ctg gag gct      7209
Ser Pro  Leu Leu Ala Cys Ala  Thr Trp Arg Ser Leu  Leu Glu Ala
    2390                 2395                 2400

gag ggt  ttt gcg ggg ctg agc  gtg cac agg gcg caa  ccc gat gcc      7254
Glu Gly  Phe Ala Gly Leu Ser  Val His Arg Ala Gln  Pro Asp Ala
    2405                 2410                 2415

ggg cag  gcc atc atc tgt gcc  tac agc gat ggg ata  gtg cgg caa      7299
Gly Gln  Ala Ile Ile Cys Ala  Tyr Ser Asp Gly Ile  Val Arg Gln
    2420                 2425                 2430

gcc agt  acg atc gag gtt gcg  cgg aat gaa aaa gta  acc gtt ccg      7344
Ala Ser  Thr Ile Glu Val Ala  Arg Asn Glu Lys Val  Thr Val Pro
    2435                 2440                 2445

tcg cag  ccg gcg gaa gcc ggg  gaa tcg ccg ctg gat  ctg gtc aaa      7389
Ser Gln  Pro Ala Glu Ala Gly  Glu Ser Pro Leu Asp  Leu Val Lys
    2450                 2455                 2460

aaa ctg  ctt gga cgc att ctg  aaa atg gat ccg gcc  aca ctc gat      7434
Lys Leu  Leu Gly Arg Ile Leu  Lys Met Asp Pro Ala  Thr Leu Asp
    2465                 2470                 2475
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acc agc  cac ccg ctg gag tac  tac ggt gtc gat tcg  atc gtg gcg      7479
Thr Ser  His Pro Leu Glu Tyr  Tyr Gly Val Asp Ser  Ile Val Ala
    2480                 2485                 2490

atc gaa  ctg gct atg gca ctg  cgc gag aca ttc ccg  ggt ttt gaa      7524
Ile Glu  Leu Ala Met Ala Leu  Arg Glu Thr Phe Pro  Gly Phe Glu
    2495                 2500                 2505

gtc agc  gag ctg ttt gaa acg  caa tcc atc gat acc  ttg ttg ggc      7569
Val Ser  Glu Leu Phe Glu Thr  Gln Ser Ile Asp Thr  Leu Leu Gly
    2510                 2515                 2520

tct ctt  gag cag gct cct ctc  ctt gct acc ctc aca  gct ccg ccg      7614
Ser Leu  Glu Gln Ala Pro Leu  Leu Ala Thr Leu Thr  Ala Pro Pro
    2525                 2530                 2535

caa caa  gac atg ctg cag cag  ctg aaa caa ctg ctg  gcg cgt acg      7659
Gln Gln  Asp Met Leu Gln Gln  Leu Lys Gln Leu Leu  Ala Arg Thr
    2540                 2545                 2550

ctg aag  ctg gac att acg cag  atc gac acg agc aag  acg ctg gag      7704
Leu Lys  Leu Asp Ile Thr Gln  Ile Asp Thr Ser Lys  Thr Leu Glu
    2555                 2560                 2565

agc tat  ggt gtc gac tcc atc  gtc atc atc gaa tta  gcc aac gcc      7749
Ser Tyr  Gly Val Asp Ser Ile  Val Ile Ile Glu Leu  Ala Asn Ala
    2570                 2575                 2580

ttg cgt  gag cgc tat ccg agc  ttg gac gcg tca cag  ctg atg gaa      7794
Leu Arg  Glu Arg Tyr Pro Ser  Leu Asp Ala Ser Gln  Leu Met Glu
    2585                 2590                 2595

acc tta  tcg atc gac cgg ctg  gtt gcc caa tgg cag  gca acg gag      7839
Thr Leu  Ser Ile Asp Arg Leu  Val Ala Gln Trp Gln  Ala Thr Glu
    2600                 2605                 2610

ccc gcc  gta ccg gca gag cca  aca gcg gaa ccg ccg  gta gcc gac      7884
Pro Ala  Val Pro Ala Glu Pro  Thr Ala Glu Pro Pro  Val Ala Asp
    2615                 2620                 2625

gaa gac  gcc gct gcc atc atc  gga ctg gcc ggc cgc  ttt cca ggc      7929
Glu Asp  Ala Ala Ala Ile Ile  Gly Leu Ala Gly Arg  Phe Pro Gly
    2630                 2635                 2640

gcg gac  acg ttg gag gag ttc  tgg aac aac ctg cgc  aac ggc caa      7974
Ala Asp  Thr Leu Glu Glu Phe  Trp Asn Asn Leu Arg  Asn Gly Gln
    2645                 2650                 2655

agc agt  atg gga gag gtg cca  ggc gag cgc tgg gat  cac cag cac      8019
Ser Ser  Met Gly Glu Val Pro  Gly Glu Arg Trp Asp  His Gln His
    2660                 2665                 2670

tac ttc  gac agt gaa cgc cag  gca ccg ggc aag acg  tat agc cgc      8064
Tyr Phe  Asp Ser Glu Arg Gln  Ala Pro Gly Lys Thr  Tyr Ser Arg
    2675                 2680                 2685

tgg ggt  gcg ttt ctg agg gac  ata gac ggc ttc gat  gca gcc ttc      8109
Trp Gly  Ala Phe Leu Arg Asp  Ile Asp Gly Phe Asp  Ala Ala Phe
    2690                 2695                 2700

ttt gaa  tgg ccc gac agc gtc  gcg ctg gaa tcg gat  ccg caa gcg      8154
Phe Glu  Trp Pro Asp Ser Val  Ala Leu Glu Ser Asp  Pro Gln Ala
    2705                 2710                 2715
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cgg ata  ttt cta gag cag gcc  tat gcc ggg atc gaa  gat gcc ggc      8199
Arg Ile  Phe Leu Glu Gln Ala  Tyr Ala Gly Ile Glu  Asp Ala Gly
    2720                 2725                 2730

tac acg  cct ggc tcg ctc agc  aag agc caa cgc gta  ggt gta ttc      8244
Tyr Thr  Pro Gly Ser Leu Ser  Lys Ser Gln Arg Val  Gly Val Phe
    2735                 2740                 2745

gta ggt  gtg atg aat ggt tac  tac agc ggc gga gcg  cgc ttc tgg      8289
Val Gly  Val Met Asn Gly Tyr  Tyr Ser Gly Gly Ala  Arg Phe Trp
    2750                 2755                 2760
caa atc  gcc aac cgc gtg tcg  tac cag ttc gat ttt  cgc ggg cca      8334
Gln Ile  Ala Asn Arg Val Ser  Tyr Gln Phe Asp Phe  Arg Gly Pro
    2765                 2770                 2775

agc ctg  gcg gtg gat acc gcc  tgt tcg gct tcg ctc  acc gcg atc      8379
Ser Leu  Ala Val Asp Thr Ala  Cys Ser Ala Ser Leu  Thr Ala Ile
    2780                 2785                 2790

cac ctg  gcg ctg gaa agc ctg  cgc agc ggc agt tgc  gag gtc gca      8424
His Leu  Ala Leu Glu Ser Leu  Arg Ser Gly Ser Cys  Glu Val Ala
    2795                 2800                 2805

ctg gcc  ggt ggc gtg aat ctg  ctg gtc gat ccg cag  caa tat ctt      8469
Leu Ala  Gly Gly Val Asn Leu  Leu Val Asp Pro Gln  Gln Tyr Leu
    2810                 2815                 2820

aat ttg  gct ggc gcc gcg atg  ctc tcc gcc ggc gcc  agc tgt cgg      8514
Asn Leu  Ala Gly Ala Ala Met  Leu Ser Ala Gly Ala  Ser Cys Arg
    2825                 2830                 2835

ccg ttc  ggc gag gcc gcg gac  ggt ttc gtg gcc ggc  gaa gcc tgc      8559
Pro Phe  Gly Glu Ala Ala Asp  Gly Phe Val Ala Gly  Glu Ala Cys
    2840                 2845                 2850

ggc gtg  gtg ctg ctc aag ccg  ctc aag caa gcg agg  gcc gat ggc      8604
Gly Val  Val Leu Leu Lys Pro  Leu Lys Gln Ala Arg  Ala Asp Gly
    2855                 2860                 2865

gat gtg  atc cat gcc gta atc  agg ggc agc atg atc  aat gcc ggt      8649
Asp Val  Ile His Ala Val Ile  Arg Gly Ser Met Ile  Asn Ala Gly
    2870                 2875                 2880

ggg cac  acc agc gcg ttc tcc  tcg cct aac cct gcc  gcc cag gcc      8694
Gly His  Thr Ser Ala Phe Ser  Ser Pro Asn Pro Ala  Ala Gln Ala
    2885                 2890                 2895

gaa gtc  gtg cgg cag gcc ttg  cag cgc gcg ggc gtg  gcg ccc gat      8739
Glu Val  Val Arg Gln Ala Leu  Gln Arg Ala Gly Val  Ala Pro Asp
    2900                 2905                 2910

tcg atc  agc tac atc gag gcg  cat ggc acc ggc acc  gta cta ggc      8784
Ser Ile  Ser Tyr Ile Glu Ala  His Gly Thr Gly Thr  Val Leu Gly
    2915                 2920                 2925

gat gca  gtg gag ttg ggt gct  ttg aat aaa gtg ttc  gac aag cgc      8829
Asp Ala  Val Glu Leu Gly Ala  Leu Asn Lys Val Phe  Asp Lys Arg
    2930                 2935                 2940

gcg gcg  cca tgc ccg atc ggc  tcg ctg aag gcg aac  atc ggc cat      8874
Ala Ala  Pro Cys Pro Ile Gly  Ser Leu Lys Ala Asn  Ile Gly His
    2945                 2950                 2955
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gcc gaa  agc gcc gcg ggc atc  gcc ggc ctg gcc aag  ctg gta ttg      8919
Ala Glu  Ser Ala Ala Gly Ile  Ala Gly Leu Ala Lys  Leu Val Leu
    2960                  2965                  2970

cag ttc  agg cat ggc gag ttg  gtg cct agt ctg aat  gcg ttt ccc      8964
Gln Phe  Arg His Gly Glu Leu  Val Pro Ser Leu Asn  Ala Phe Pro
    2975                  2980                  2985

ttg aat  ccc tat att gag ttc  ggt cgc ttc cag gta  caa cag cag      9009
Leu Asn  Pro Tyr Ile Glu Phe  Gly Arg Phe Gln Val  Gln Gln Gln
    2990                  2995                  3000

ccg gca  ccg tgg ccg cgc cgt  ggc gcc cag ccg cgg  cgc gcc ggg      9054
Pro Ala  Pro Trp Pro Arg Arg  Gly Ala Gln Pro Arg  Arg Ala Gly
    3005                  3010                  3015

tta tct  gcc ttc ggt gct ggc  gga tcg aat gcg cac  cta gtg gta      9099
Leu Ser  Ala Phe Gly Ala Gly  Gly Ser Asn Ala His  Leu Val Val
    3020                  3025                  3030

gag gaa  gct ccg gct atg gct  ccc ggg gtc tcg atc  agc gcc agc      9144
Glu Glu  Ala Pro Ala Met Ala  Pro Gly Val Ser Ile  Ser Ala Ser
    3035                  3040                  3045

tct cca  gcc ttg atc gtg ctt  tcg gcg cga acg ctg  cct gcc ttg      9189
Ser Pro  Ala Leu Ile Val Leu  Ser Ala Arg Thr Leu  Pro Ala Leu
    3050                  3055                  3060

caa cag  cgt gct cgc gat ctg  ctc gtc tgg atg caa  gcg cgg cag      9234
Gln Gln  Arg Ala Arg Asp Leu  Leu Val Trp Met Gln  Ala Arg Gln
    3065                  3070                  3075

gtg gat  gac gtc atg ctg gcc  gac gtt gct tat acg  ctg cac ttg      9279
Val Asp  Asp Val Met Leu Ala  Asp Val Ala Tyr Thr  Leu His Leu
    3080                  3085                  3090

ggc cgc  gtc gcg atg gag caa  cgc ctg gct ttt acc  gct ggc tcg      9324
Gly Arg  Val Ala Met Glu Gln  Arg Leu Ala Phe Thr  Ala Gly Ser
    3095                  3100                  3105

gct gcc  gag ttg agc gag aaa  tta cag gct tac ctg  ggc cat gcg      9369
Ala Ala  Glu Leu Ser Glu Lys  Leu Gln Ala Tyr Leu  Gly His Ala
    3110                  3115                  3120

att cgg  gcc gac atc tat ctg  agc gag gac acg ccc  ggc aaa ccg      9414
Ile Arg  Ala Asp Ile Tyr Leu  Ser Glu Asp Thr Pro  Gly Lys Pro
    3125                  3130                  3135

gca ggc  gct ccg atc gtg gcc  gag gaa gat ctg ctc  acg ctg atg      9459
Ala Gly  Ala Pro Ile Val Ala  Glu Glu Asp Leu Leu  Thr Leu Met
    3140                  3145                  3150

gat gcc  tgg atc gaa aag ggc  cag tac ggt cgt ttg  ctg gag tac      9504
Asp Ala  Trp Ile Glu Lys Gly  Gln Tyr Gly Arg Leu  Leu Glu Tyr
    3155                  3160                  3165

tgg acc  aag ggc caa ccg atc  gac tgg aac aaa ctc  tat tgg cgc      9549
Trp Thr  Lys Gly Gln Pro Ile  Asp Trp Asn Lys Leu  Tyr Trp Arg
    3170                  3175                  3180

aag ctg  tat gcg gac gga cgg  ccg cgg cgg atc agc  ctg ccc acc      9594
Lys Leu  Tyr Ala Asp Gly Arg  Pro Arg Arg Ile Ser  Leu Pro Thr
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    3185                3190                3195

tat ccg  ttc gag cac cgg cgt  tat tgg caa acg ccg  gtg ccg ggc      9639
Tyr Pro  Phe Glu His Arg Arg  Tyr Trp Gln Thr Pro  Val Pro Gly
    3200                3205                3210

gag cga  agc ctg cac gcc acc  gcg cca gct act cgg  gaa acg gtt      9684
Glu Arg  Ser Leu His Ala Thr  Ala Pro Ala Thr Arg  Glu Thr Val
    3215                3220                3225

gcg gtt  ggt gcc atg ccg gat  ccg gcc ggc gct acg  gtg caa gcc      9729
Ala Val  Gly Ala Met Pro Asp  Pro Ala Gly Ala Thr  Val Gln Ala
    3230                3235                3240

cgg ttg  tgc gcc ttg tgc caa  gtg ttg ttg ggc aaa  ccg gtc acg      9774
Arg Leu  Cys Ala Leu Cys Gln  Val Leu Leu Gly Lys  Pro Val Thr
    3245                3250                3255

gcc cag  atg gat ttc ttt gcc  gtc ggc ggc cat tcg  gtg ctg gcg      9819
Ala Gln  Met Asp Phe Phe Ala  Val Gly Gly His Ser  Val Leu Ala
    3260                3265                3270

atc caa  ttg gtc tcg cgc atc  cgc aaa agc ttc ggg  gtg gag tat      9864
Ile Gln  Leu Val Ser Arg Ile  Arg Lys Ser Phe Gly  Val Glu Tyr
    3275                3280                3285

ccg gtc  agc gct ttg ttc gaa  tcg gcg ctg ttg tcg  gac atg gcg      9909
Pro Val  Ser Ala Leu Phe Glu  Ser Ala Leu Leu Ser  Asp Met Ala
    3290                3295                3300

cgg cag  atc gaa caa ttg cgg  gtg aac gga gtc gcc  aag cgc atg      9954
Arg Gln  Ile Glu Gln Leu Arg  Val Asn Gly Val Ala  Lys Arg Met
    3305                3310                3315

ccg gcg  ttg ttg cct gcc ggg  cgc gtg ggc gcg att  cct gcg act      9999
Pro Ala  Leu Leu Pro Ala Gly  Arg Val Gly Ala Ile  Pro Ala Thr
    3320                3325                3330

tat gca  cag gag cgc cta tgg  ctc gtc cac gaa cat  atg agt gag     10044
Tyr Ala  Gln Glu Arg Leu Trp  Leu Val His Glu His  Met Ser Glu
    3335                3340                3345

caa cgc  agt agt tac aac atc  acc ttt gcc atg cac  ttc aga ggc     10089
Gln Arg  Ser Ser Tyr Asn Ile  Thr Phe Ala Met His  Phe Arg Gly
    3350                3355                3360

gtc gac  ttc cgt gct gaa gcg  atg cgt gcc gca ttg  aac gcg ctg     10134
Val Asp  Phe Arg Ala Glu Ala  Met Arg Ala Ala Leu  Asn Ala Leu
    3365                3370                3375

gtg gtg  cgg cac gaa gtg ctg  cgc aca cgc ttt ctt  tcg gag gac     10179
Val Val  Arg His Glu Val Leu  Arg Thr Arg Phe Leu  Ser Glu Asp
    3380                3385                3390

ggg cag  ctg caa cag gtg atc  gct gcc tcg ttg acg  ttg gag gtg     10224
Gly Gln  Leu Gln Gln Val Ile  Ala Ala Ser Leu Thr  Leu Glu Val
    3395                3400                3405

ccg gta  aga gag atg tcg gtc  gag gag gtc gac ctg  ctg ctg gcc     10269
Pro Val  Arg Glu Met Ser Val  Glu Glu Val Asp Leu  Leu Leu Ala
    3410                3415                3420

gcg agc  acg cgg gag act ttc  gat ctg cgg cag ggg  ccc ttg ttc     10314
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Ala Ser  Thr Arg Glu Thr Phe  Asp Leu Arg Gln Gly  Pro Leu Phe
    3425                 3430                 3435

aag gca  cgc atc ctg cgc gtg  gcg gcc gat cac cat  gtg gtg ttg     10359
Lys Ala  Arg Ile Leu Arg Val  Ala Ala Asp His His  Val Val Leu
    3440                 3445                 3450
agc agc  atc cac cac atc att  tcc gac ggc tgg tcg  ctg gga gtg     10404
Ser Ser  Ile His His Ile Ile  Ser Asp Gly Trp Ser  Leu Gly Val
    3455                 3460                 3465

ttc aac  cgt gac ctg cac cag  ctg tac gag gcg tgt  ttg cgc ggc     10449
Phe Asn  Arg Asp Leu His Gln  Leu Tyr Glu Ala Cys  Leu Arg Gly
    3470                 3475                 3480

acg ccc  ccc aca ctg ccg acg  ctg gcg gtg cag tat  gcc gac tac     10494
Thr Pro  Pro Thr Leu Pro Thr  Leu Ala Val Gln Tyr  Ala Asp Tyr
    3485                 3490                 3495

gcg ctg  tgg caa cgg caa tgg  gag ctg gcg gct ccg  ctg tcg tac     10539
Ala Leu  Trp Gln Arg Gln Trp  Glu Leu Ala Ala Pro  Leu Ser Tyr
    3500                 3505                 3510

tgg acg  cgg gca ctg gaa ggc  tac gac gac ggc ctg  gac ttg ccc     10584
Trp Thr  Arg Ala Leu Glu Gly  Tyr Asp Asp Gly Leu  Asp Leu Pro
    3515                 3520                 3525

tac gac  cgg ccg cgc ggc gcc  acg cgg gcg tgg cgg  gca ggg ctg     10629
Tyr Asp  Arg Pro Arg Gly Ala  Thr Arg Ala Trp Arg  Ala Gly Leu
    3530                 3535                 3540

gtc aaa  cac cgc tat ccg ccg  caa ctg gcc cag cag  ttg gcg gcc     10674
Val Lys  His Arg Tyr Pro Pro  Gln Leu Ala Gln Gln  Leu Ala Ala
    3545                 3550                 3555

tac agc  caa cag tac caa gcg  acg ctg ttc atg agc  ctg ctg gca     10719
Tyr Ser  Gln Gln Tyr Gln Ala  Thr Leu Phe Met Ser  Leu Leu Ala
    3560                 3565                 3570

ggc ctg  gcg ttg gtg ctg ggc  cgt tac gcc gat cgc  aag gac gtg     10764
Gly Leu  Ala Leu Val Leu Gly  Arg Tyr Ala Asp Arg  Lys Asp Val
    3575                 3580                 3585

tgc atc  ggc gcg acg gtc tcc  ggc cgc gac cag ctg  gag ctg gaa     10809
Cys Ile  Gly Ala Thr Val Ser  Gly Arg Asp Gln Leu  Glu Leu Glu
    3590                 3595                 3600

gag ctg  atc ggc ttt ttc atc  aat att ttg ccg ctg  cgg gtg gac     10854
Glu Leu  Ile Gly Phe Phe Ile  Asn Ile Leu Pro Leu  Arg Val Asp
    3605                 3610                 3615

ctg tcg  ggg gat ccg tgc ctg  gag gag gtg ctg ctg  cgc acg cgt     10899
Leu Ser  Gly Asp Pro Cys Leu  Glu Glu Val Leu Leu  Arg Thr Arg
    3620                 3625                 3630

caa gtg  gta ctg gat ggc ttc  gcg cac cag tcg gtg  ccg ttc gag     10944
Gln Val  Val Leu Asp Gly Phe  Ala His Gln Ser Val  Pro Phe Glu
    3635                 3640                 3645

cac gtg  ttg cag gcg ctg cgg  cgt cag cgc gac agt  agc cag atc     10989
His Val  Leu Gln Ala Leu Arg  Arg Gln Arg Asp Ser  Ser Gln Ile
    3650                 3655                 3660

ccg ctg  gtg ccg gtg atg ctg  cga cac cag aac ttc  ccg acg cag     11034
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Pro Leu  Val Pro Val Met Leu  Arg His Gln Asn Phe  Pro Thr Gln
    3665                 3670                 3675

gag att  ggc gat tgg ccc gag  gga gtg cgg ctg acg  cag atg gag     11079
Glu Ile  Gly Asp Trp Pro Glu  Gly Val Arg Leu Thr  Gln Met Glu
    3680                 3685                 3690

ctg ggg  ctg gac cgt agc acg  ccg agc gag ctg gat  tgg cag ttc     11124
Leu Gly  Leu Asp Arg Ser Thr  Pro Ser Glu Leu Asp  Trp Gln Phe
    3695                 3700                 3705

tac ggc  gac ggc agc tcg ctg  gag ctg acg ctg gaa  tac gcg cag     11169
Tyr Gly  Asp Gly Ser Ser Leu  Glu Leu Thr Leu Glu  Tyr Ala Gln
    3710                 3715                 3720

gac ctc  ttc gac gaa gcg acg  gtg cgg cgg atg atc  gca cac cac     11214
Asp Leu  Phe Asp Glu Ala Thr  Val Arg Arg Met Ile  Ala His His
    3725                 3730                 3735

cag cag  gcg ttg gag gcg atg  gtg agc cgg cca cag  ctg cgg gtg     11259
Gln Gln  Ala Leu Glu Ala Met  Val Ser Arg Pro Gln  Leu Arg Val
    3740                 3745                 3750

ggc aag  tgg gac atg ctg acg  gcc gaa gag cgc cgg  ctg ttt gcc     11304
Gly Lys  Trp Asp Met Leu Thr  Ala Glu Glu Arg Arg  Leu Phe Ala
    3755                 3760                 3765

gcg cta  aat gcg aca ggt acg  cca cgg gag tgg ccc  agt ctg gcg     11349
Ala Leu  Asn Ala Thr Gly Thr  Pro Arg Glu Trp Pro  Ser Leu Ala
    3770                 3775                 3780

cag cag  ttc gaa cgg cag gcg  cag gcg acg ccg cag  gcc ata gca     11394
Gln Gln  Phe Glu Arg Gln Ala  Gln Ala Thr Pro Gln  Ala Ile Ala
    3785                 3790                 3795

tgc gtg  agc gat ggg cag tcg  tgg agc tat gcg cag  ttg gag gcg     11439
Cys Val  Ser Asp Gly Gln Ser  Trp Ser Tyr Ala Gln  Leu Glu Ala
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cgc gcc  aac cag ctg gca cag  gcg ctg cgt ggg cag  ggc gcg ggc     11484
Arg Ala  Asn Gln Leu Ala Gln  Ala Leu Arg Gly Gln  Gly Ala Gly
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Arg Asp  Val Arg Val Ala Val  Gln Ser Ala Arg Thr  Pro Glu Leu
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ctg atg  gcc ttg ctg gcg atc  ttc aag gcc ggt gca  tgc tat gtg     11574
Leu Met  Ala Leu Leu Ala Ile  Phe Lys Ala Gly Ala  Cys Tyr Val
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ccg atc  gat ccg gcc tac ccg  gcg gcc tac cgc gag  cag atc ctg     11619
Pro Ile  Asp Pro Ala Tyr Pro  Ala Ala Tyr Arg Glu  Gln Ile Leu
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gcc gag  gtg cag gtg tcg atc  gtg ctg gag caa gac  gag ctg gcg     11664
Ala Glu  Val Gln Val Ser Ile  Val Leu Glu Gln Asp  Glu Leu Ala
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ctg gac  gag caa ggg cag ttc  cac aat ccg cgt tgg  cgc gag caa     11709
Leu Asp  Glu Gln Gly Gln Phe  His Asn Pro Arg Trp  Arg Glu Gln
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Cys Val  Met Val Thr Ser Gly  Ser Thr Gly Arg Pro  Lys Gly Val
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Met Val  Pro Tyr Ala Gln Leu  His Asn Trp Leu His  Ala Gly Trp
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Gln Arg  Ser Ala Phe Glu Ala  Gly Glu Arg Val Leu  Gln Lys Thr
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Ser Ile  Ala Phe Ala Val Ser  Val Lys Glu Leu Leu  Ser Gly Leu
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Leu Ala  Gly Val Glu Gln Val  Met Leu Pro Asp Glu  Gln Val Lys
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Asp Ser  Leu Ala Leu Ala Arg  Ala Ile Glu Gln Trp  Gln Val Thr
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Arg Leu  Tyr Leu Val Pro Ser  His Leu Gln Ala Leu  Leu Asp Ala
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Thr Gln  Gly Arg Asp Gly Leu  Leu His Ser Leu Arg  His Val Val
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Thr Ala  Gly Glu Ala Leu Pro  Ser Ala Val Arg Glu  Thr Val Arg
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Val Arg  Leu Pro Gln Val Gln  Leu Trp Asn Asn Tyr  Gly Cys Thr
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Glu Leu  Asn Asp Ala Thr Tyr  His Arg Ser Asp Thr  Val Ala Pro
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gga acg  ttt gtg ccg atc ggc  gca ccg atc gcc aac  acc gag gta      12294
Gly Thr  Phe Val Pro Ile Gly  Ala Pro Ile Ala Asn  Thr Glu Val
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Tyr Val  Leu Asp Arg Gln Leu  Arg Gln Val Pro Ile  Gly Val Met
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Gly Glu  Leu His Val His Ser  Val Gly Met Ala Arg  Gly Tyr Trp
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Asn Arg  Pro Gly Leu Thr Ala  Ser Arg Phe Ile Ala  His Pro Tyr
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Arg Arg  Leu Ala Asp Gly Thr  Leu Glu Tyr Leu Gly  Arg Gln Asp
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Phe Glu  Val Lys Val Arg Gly  His Arg Val Asp Thr  Arg Gln Val
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Glu Ala  Ala Leu Arg Ala Gln  Pro Ala Val Ala Glu  Ala Val Val
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Ser Gly  His Arg Val Asp Gly  Asp Met Gln Leu Val  Ala Tyr Val
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Val Ala  Arg Glu Gly Gln Ala  Pro Ser Ala Gly Glu  Leu Lys Gln
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Gln Leu  Ser Ala Gln Leu Pro  Thr Tyr Met Leu Pro  Thr Val Tyr
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Arg Leu  Ala Leu Pro Ala Pro  Gln Val Val His Ala  Gln Glu Tyr
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Val Ala  Pro Arg Asn Glu Ala  Glu Gln Arg Leu Ala  Ala Leu Phe
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Ala Glu  Val Leu Arg Val Glu  Gln Val Gly Ile His  Asp Asn Phe
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ttc gcc  ttg ggt ggg cac tcg  ctg tct gca tcg caa  ctg atc tcg      12969
Phe Ala  Leu Gly Gly His Ser  Leu Ser Ala Ser Gln  Leu Ile Ser
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Arg Ile  Arg Gln Ser Phe His  Val Asp Leu Pro Leu  Ser Arg Ile
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Phe Glu  Ala Pro Thr Ile Glu  Gly Leu Val Arg Gln  Leu Ala Leu
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cct agt  gaa ggc ggc gtg gcc  agc atc gcc agg gta  gcg cga aac      13104
Pro Ser  Glu Gly Gly Val Ala  Ser Ile Ala Arg Val  Ala Arg Asn
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Ala Leu  Arg Leu Arg Gly Pro  Leu Ser Val Glu Ala  Met Arg Ala
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Ala Leu  Arg Ala Leu Val Leu  Arg His Glu Ile Leu  Arg Thr Arg
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Phe Val  Leu Pro Thr Gly Ala  Ser Glu Pro Val Gln  Val Ile Asp
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Glu His  Ser Asp Phe Gln Leu  Ser Val Gln Leu Val  Glu Asp Thr
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Leu Ala  Asn Gly Pro Leu Phe  Ile Ala Cys Leu Leu  Gln Leu Asp
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Glu Gln  Glu His Val Leu Leu  Ile Gly Met His His  Leu Ile Tyr
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Asp Ala  Trp Ser Gln Phe Thr  Val Met Asn Arg Asp  Leu Arg Val
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ctg tat  cac cgc cac ctc gga  ctt gcc ggc gga gat  ctg ccg gaa     13599
Leu Tyr  His Arg His Leu Gly  Leu Ala Gly Gly Asp  Leu Pro Glu
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Leu Pro  Ile Gln Tyr Ala Asp  Tyr Ala Ile Trp Gln  Arg Ala Gln
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Asn Leu  Asp Ala Gln Leu Ala  Tyr Trp Gln Ala Met  Leu His Asp
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Tyr Asp  Asp Gly Leu Glu Leu  Pro Tyr Asp Tyr Pro  Arg Pro Arg
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aat cgc  acc tgg cac gca gcg  gtc tac aca cac acc  tat ccg gct     13779
Asn Arg  Thr Trp His Ala Ala  Val Tyr Thr His Thr  Tyr Pro Ala
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gaa ctg  gta cag cgc ttt gcc  ggc ttc gta cag gcg  cat cag tcg     13824
Glu Leu  Val Gln Arg Phe Ala  Gly Phe Val Gln Ala  His Gln Ser
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Gly Arg  Thr His Leu Glu Leu  Glu Asn Leu Ile Gly  Phe Phe Ile
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Asn Ile  Leu Pro Leu Arg Leu  Arg Leu Asp Gly Asp  Pro Asp Val
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Arg His  Gln Asn Phe Pro Asp  Thr Ile Gly Asp Trp  Ser Asp Gly
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Ile Arg  Thr Glu Val Ile Gln  Arg Asp Leu Arg Ala  Thr Pro Asn
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Glu Met  Asp Leu Gln Phe Phe  Gly Asp Gly Thr Gly  Leu Ser Val
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Thr Val  Glu Tyr Ala Ala Glu  Leu Phe Ser Glu Ala  Thr Ile Arg
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Arg Leu  Ile His His His Gln  Leu Val Leu Glu Gln  Met Leu Ala
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Thr Tyr  Gln Glu Ala Gly Val  Glu Pro Ala Ser Val  Gly Tyr Val
    1025                1030                1035
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Glu Leu His Gly Thr Gly Thr Ser Leu Gly Asp Pro Ile Glu Ile
1040 1045 1050

Gln Ala Leu Lys Glu Ala Phe Ile Ala Leu Gly Ala Gln Ala Ala
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Pro Ser Asn Cys Gly Ile Gly Ser Val Lys Ser Ala Leu Gly His
1070 1075 1080

Leu Glu Ala Ala Ala Gly Leu Thr Gly Leu Ile Lys Val Leu Leu
1085 1090 1095

Met Leu Lys His Gly Glu Gln Ala Gly Thr Arg His Phe Ser Thr
1100 1105 1110

Leu Asn Pro Leu Ile Asp Leu Arg Gly Thr Ser Phe Glu Val Val
1115 1120 1125

Ala Gln His Arg Ala Trp Pro Ser Gln Val Gly Ile His Gly Thr
1130 1135 1140

Leu Leu Pro Arg Arg Ala Gly Ile Ser Ser Phe Gly Phe Gly Gly
1145 1150 1155

Ala Asn Ala His Ala Ile Val Glu Glu His Val Ile Ala Thr Pro
1160 1165 1170

Pro Ser Thr Ser Ser Ala Gly Gly Pro Val Gly Ile Val Leu Ser
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Ala Gly Ser Glu Ala Val Leu Arg Gln Gln Val Leu Ala Leu Ser
1190 1195 1200

Ala Trp Leu Arg Gln Gln Ser Pro Thr Pro Ala Gln Met Ile Asp
1205 1210 1215

Val Ala Tyr Thr Leu Gln Val Gly Arg Ala Ala Leu Ser His Arg
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Leu Ala Phe Ser Ala Thr Asp Ala Glu Gln Ala Leu Ala Arg Leu
1235 1240 1245

Glu Gly Arg Leu Ala Gly Val Met Asp Ala Glu Val His His Gly
1250 1255 1260

Val Val Asp Ala Ala Ala Thr Ala Pro Glu His Gly Arg Gln Thr
1265 1270 1275

Arg Glu Gly Leu Ala Gly Leu Leu Arg Ala Trp Thr Gln Gly Val
1280 1285 1290

Arg Val Asp Trp Ser Ala Leu Tyr Gly Ile Gln Arg Pro Gln Arg
1295 1300 1305

Val Ser Leu Pro Val Tyr Pro Phe Ala Arg Glu Arg Tyr Trp Leu
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Pro Gly Gln Ala Met His Ala Ala Ala Asp Ala His Pro Met Leu
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Gln Leu Leu His Ala Asn Ala Lys Leu His Arg Tyr Ala Leu Arg
1340 1345 1350

Arg Ser Gly Cys Ala Ser Phe Leu Val Asp His Cys Val Asp Gly
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Arg Gln Val Leu Pro Ala Ala Val Gln Leu Glu Leu Val Arg Ala
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Val Ala Gln Arg Val Met Ala Gln Asp Glu Gly Cys Ile Glu Leu
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Ala Gln Val Ala Phe Leu His Pro Leu Met Met Glu Glu Thr Glu
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Leu Glu Val Glu Ile Glu Leu Ser Lys Ser Asp Gln Asp Glu Phe
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Asp Phe Gln Leu His Asp Ala His Arg Gln Gln Val Phe Ser Gln
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Gly His Val Arg Arg Arg Val Tyr Thr Ala Thr Pro Arg Leu Asp
1445 1450 1455

Leu Ala Gln Leu Gln Lys Leu Cys Ala Glu Arg Val Leu Ser Gly
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Glu Asp Cys Tyr Ala His Phe Thr Ala Cys Gly Leu Gln Leu Gly
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Asp Arg Leu Lys Ser Val Gln Ser Ile Gly Cys Gly Arg Asn Gly
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Glu Gly Glu Pro Ile Ala Leu Gly Val Leu Arg Leu Pro Pro Ser

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Ser Val Glu Asp Ser His Val Leu Pro Pro Ser Leu Leu Asp Gly
1520 1525 1530

Ala Leu Gln Cys Ser Leu Gly Leu Gln Arg Asp Val Glu His Ile
1535 1540 1545

Ala Met Pro Tyr Thr Leu Glu Arg Met Thr Val His Ala Pro Ile
1550 1555 1560

Pro Pro Glu Ala Trp Val Leu Leu Arg His Gly His Ala Ala Arg
1565 1570 1575

Gln Ser Leu Asp Ile Asp Leu Leu Asp Ser Glu Gly Arg Val Cys
1580 1585 1590

Val Ser Leu Gly Asn Tyr Thr Gly Arg Ala Pro Lys Ala Val Ser
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Ala Val Arg Ala Leu Val Leu Ala Pro Val Trp Gln Ala Leu Thr
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Glu Thr Ala Pro Ala Trp Pro Asp Pro Ala Glu Arg Ile Val Thr
1625 1630 1635

Val Gly Asp Asp Ala Trp Arg Ser His Phe Gly Phe Asp Glu Pro
1640 1645 1650

Ala Leu Ser Leu Glu Asp Ser Val Glu Val Ile Ala Thr Arg Leu
1655 1660 1665

Gly Gln Ser Gly Lys Phe Asp His Leu Val Trp Ile Val Pro Ile
1670 1675 1680

Ala Glu Ser Glu Thr Asp Ile Ala Ala Gln Gly Ser Ala Ala Ile
1685 1690 1695

Ala Gly Phe Arg Leu Val Lys Ala Leu Leu Ala Leu Gly Tyr Ala
1700 1705 1710

His Arg Pro Leu Gly Leu Thr Val Leu Thr Arg Gln Ala Leu Thr
1715 1720 1725

Arg Gln Pro Ser His Ala Ala Val His Gly Leu Ile Gly Thr Leu
1730 1735 1740

Ala Lys Glu Tyr Cys Asn Trp Lys Ile Arg Leu Leu Asp Leu Pro

1745 1750 1755

Ser Val Lys Ser Trp Pro Gln Trp Glu Gln Leu Arg Ser Leu Pro
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Trp His Ala Gln Gly Glu Ala Leu Ile Gly Arg Gly Thr Cys Trp
1775 1780 1785

Tyr Arg Arg Gln Leu Cys Glu Val Leu Pro Leu Pro Ser Leu Glu
1790 1795 1800

Pro Pro Pro Tyr Arg Val Gly Gly Val Tyr Val Val Ile Gly Gly
1805 1810 1815

Ala Gly Gly Leu Gly Glu Val Leu Ser Glu His Leu Ile Arg Thr
1820 1825 1830

Tyr Asp Ala Gln Leu Ile Trp Ile Gly Arg Arg Val Leu Asp Glu
1835 1840 1845

Gly Ile Ala Arg Lys Gln Thr Arg Leu Ala Ser Leu Gly Arg Ala
1850 1855 1860

Pro His Tyr Ile Ser Ala Asp Ala Ser Asp Pro Ala Ala Leu Gln
1865 1870 1875

Ala Ala His Asn Glu Ile Val Asn Leu His Gly Gln Pro His Gly
1880 1885 1890

Leu Ile Leu Ser Asn Ile Val Leu Lys Asp Ala Ser Leu Ala Arg
1895 1900 1905

Met Glu Glu Ala Asp Phe Arg Asp Val Leu Ala Ala Lys Leu Asp
1910 1915 1920

Val Ser Val Cys Ala Ala Gln Val Phe Gly Thr Ala Pro Leu Asp
1925 1930 1935

Phe Val Leu Phe Phe Ser Ser Ile Gln Ser Thr Thr Lys Ala Ala
1940 1945 1950

Gly Gln Gly Asn Tyr Ala Ala Gly Cys Cys Tyr Val Asp Ala Phe
1955 1960 1965

Gly Glu Leu Trp Ala Arg Arg Gly Leu Arg Val Lys Thr Ile Asn
1970 1975 1980

Trp Gly Tyr Trp Gly Ser Val Gly Val Val Ala Gly Glu Asp Tyr
1985 1990 1995

Arg Arg Arg Met Ala Gln Lys His Met Ala Ser Ile Glu Gly Ala
2000 2005 2010

Glu Ala Met Gln Val Leu Ser Gln Leu Leu Cys Ala Pro Leu Gln
2015 2020 2025

Arg Leu Ala Tyr Val Lys Ile Asp Asp Ala Asn Ala Met Arg Ala
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Ser Val Asn Leu Asp Ala Arg Arg Glu Arg Glu Thr Leu Leu Ala
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2240 2245 2250

Ala Gly Ser Gly Gly Thr Thr Ala Ser Val Leu Gln Gln Leu Gln
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Ala Tyr Gly Glu His Ile Glu Glu Tyr Leu Tyr Thr Asp Leu Ser
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Pro Val Phe Leu His His Ala Glu Lys His Tyr Gln Pro Arg Ala
2285 2290 2295

Pro Tyr Leu Arg Thr Ala Cys Phe Asp Val Ala Arg Ala Pro Thr
2300 2305 2310

Ala Gln Ala Leu Glu Ser Gly Gly Tyr Asp Val Val Ile Ala Ala
2315 2320 2325

Asn Val Leu His Ala Thr Arg Asp Ile Ala Lys Thr Leu Arg Asn
2330 2335 2340

Ala Lys Ala Leu Leu Lys Pro Gly Gly Leu Leu Leu Leu Asn Glu
2345 2350 2355

Val Ile Glu Arg Ser Leu Val Leu His Leu Thr Phe Gly Leu Leu
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Glu Ser Trp Trp Leu Pro Gln Asp Lys Ile Leu Arg Leu Ala Gly
2375 2380 2385

Ser Pro Leu Leu Ala Cys Ala Thr Trp Arg Ser Leu Leu Glu Ala
2390 2395 2400

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Gly Gln Ala Ile Ile Cys Ala Tyr Ser Asp Gly Ile Val Arg Gln
2420 2425 2430

Ala Ser Thr Ile Glu Val Ala Arg Asn Glu Lys Val Thr Val Pro
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Ser Gln Pro Ala Glu Ala Gly Glu Ser Pro Leu Asp Leu Val Lys
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Lys Leu Leu Gly Arg Ile Leu Lys Met Asp Pro Ala Thr Leu Asp
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2495 2500 2505

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Glu Asp Ala Ala Ala Ile Ile Gly Leu Ala Gly Arg Phe Pro Gly
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Ala Asp Thr Leu Glu Glu Phe Trp Asn Asn Leu Arg Asn Gly Gln
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Ser Ser Met Gly Glu Val Pro Gly Glu Arg Trp Asp His Gln His
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Tyr Phe Asp Ser Glu Arg Gln Ala Pro Gly Lys Thr Tyr Ser Arg
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Trp Gly Ala Phe Leu Arg Asp Ile Asp Gly Phe Asp Ala Ala Phe
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Tyr Thr Pro Gly Ser Leu Ser Lys Ser Gln Arg Val Gly Val Phe
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Val Gly Val Met Asn Gly Tyr Tyr Ser Gly Gly Ala Arg Phe Trp
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Gly His Thr Ser Ala Phe Ser Ser Pro Asn Pro Ala Ala Gln Ala
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Gly Arg Val Ala Met Glu Gln Arg Leu Ala Phe Thr Ala Gly Ser
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Tyr Ser Gln Gln Tyr Gln Ala Thr Leu Phe Met Ser Leu Leu Ala
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Phe Glu Val Lys Val Arg Gly His Arg Val Asp Thr Arg Gln Val
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Ser Gly His Arg Val Asp Gly Asp Met Gln Leu Val Ala Tyr Val
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His Gln His Met Pro Glu Gln Arg Thr Ser Tyr Asn Gly Thr Leu
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Asp Ala Trp Ser Gln Phe Thr Val Met Asn Arg Asp Leu Arg Val
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Leu Pro Ile Gln Tyr Ala Asp Tyr Ala Ile Trp Gln Arg Ala Gln
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Gly Arg Thr His Leu Glu Leu Glu Asn Leu Ile Gly Phe Phe Ile
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Glu Met Asp Leu Gln Phe Phe Gly Asp Gly Thr Gly Leu Ser Val
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Thr Val Glu Tyr Ala Ala Glu Leu Phe Ser Glu Ala Thr Ile Arg
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<221> CDS
<222> (1)..(30)
<223> Beta-ketoacyl synthase 1 subdomain III

<400> 13
gcg ctg ggc cat cta gaa gcc gct gca ggc 30
Ala Leu Gly His Leu Glu Ala Ala Ala Gly

1 5 10

<210> 14
<211> 10
<212> PRT
<213> Xanthomonas albilineans

<400> 14

Ala Leu Gly His Leu Glu Ala Ala Ala Gly
1 5 10

<210> 15
<211> 51
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(51)
<223> Beta-ketoacyl synthase 2 subdomain I

<400> 15
ggg cca agc ctg gcg gtg gat acc gcc tgt tcg gct tcg ctc acc gcg 48
Gly Pro Ser Leu Ala Val Asp Thr Ala Cys Ser Ala Ser Leu Thr Ala
1 5 10 15

atc 51
Ile

<210> 16
<211> 17
<212> PRT
<213> Xanthomonas albilineans

<400> 16
Gly Pro Ser Leu Ala Val Asp Thr Ala Cys Ser Ala Ser Leu Thr Ala
1 5 10 15

Ile

<210> 17
<211> 30
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(30)
<223> Beta-ketoacyl synthase 2 subdomain II

<400> 17
atc gag gcg cat ggc acc ggc acc gta cta 30
Ile Glu Ala His Gly Thr Gly Thr Val Leu

1 5 10

<210> 18
<211> 10
<212> PRT
<213> Xanthomonas albilineans

<400> 18

Ile Glu Ala His Gly Thr Gly Thr Val Leu
1 5 10

<210> 19
<211> 30
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(30)
<223> Beta-ketoacyl synthase 2 subdomain III

<400> 19
cgt tct cct cgc cta acc ctg ccg ccc agg 30
Arg Ser Pro Arg Leu Thr Leu Pro Pro Arg
1 5 10

<210> 20
<211> 10
<212> PRT
<213> Xanthomonas albilineans

<400> 20

Arg Ser Pro Arg Leu Thr Leu Pro Pro Arg
1 5 10

<210> 21
<211> 93
<212> DNA
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<220>
<221> CDS
<222> (1)..(93)
<223> Beta-ketoacyl reductase domain

<400> 21
gtc tac gtc gtg atc ggc ggc gct ggc ggc ttg ggt gaa gta ttg agc 48
Val Tyr Val Val Ile Gly Gly Ala Gly Gly Leu Gly Glu Val Leu Ser
1 5 10 15

gaa cac ttg atc cgc acg tac gac gcg cag ctg atc tgg atc ggg 93
Glu His Leu Ile Arg Thr Tyr Asp Ala Gln Leu Ile Trp Ile Gly
20 25 30

<210> 22
<211> 31
<212> PRT
<213> Xanthomonas albilineans

<400> 22

Val Tyr Val Val Ile Gly Gly Ala Gly Gly Leu Gly Glu Val Leu Ser
1 5 10 15

Glu His Leu Ile Arg Thr Tyr Asp Ala Gln Leu Ile Trp Ile Gly
20 25 30

<210> 23
<211> 36
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(36)
<223> Acyl protein carrier 1 domain

<400> 23
tgc gaa ctg gcg ctg gat tcg ctc caa tgc gtg cgt 36
Cys Glu Leu Ala Leu Asp Ser Leu Gln Cys Val Arg
1 5 10

<210> 24
<211> 12
<212> PRT
<213> Xanthomonas albilineans

<400> 24

Cys Glu Leu Ala Leu Asp Ser Leu Gln Cys Val Arg
1 5 10

<210> 25
<211> 36
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(36)
<223> Acyl carrier protein 2 domain

<400> 25
gag tac tac ggt gtc gat tcg atc gtg gcg atc gaa 36
Glu Tyr Tyr Gly Val Asp Ser Ile Val Ala Ile Glu
1 5 10

<210> 26
<211> 12
<212> PRT

<213> Xanthomonas albilineans

<400> 26

Glu Tyr Tyr Gly Val Asp Ser Ile Val Ala Ile Glu
1 5 10

<210> 27
<211> 36
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(36)
<223> Acyl carrier protein 3 domain

<400> 27
gag agc tat ggt gtc gac tcc atc gtc atc atc gaa 36
Glu Ser Tyr Gly Val Asp Ser Ile Val Ile Ile Glu
1 5 10

<210> 28
<211> 12
<212> PRT
<213> Xanthomonas albilineans

<400> 28

Glu Ser Tyr Gly Val Asp Ser Ile Val Ile Ile Glu
1 5 10

<210> 29
<211> 18
<212> DNA
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<220>
<221> CDS
<222> (1)..(18)
<223> Adenylation domain subdomain I

<400> 29
tgg agc tat gcg cag ttg 18
Trp Ser Tyr Ala Gln Leu
1 5

<210> 30
<211> 6
<212> PRT
<213> Xanthomonas albilineans

<400> 30

Trp Ser Tyr Ala Gln Leu
1 5

<210> 31
<211> 33
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(33)
<223> Adenylation domain subdomain II

<400> 31
ttc aag gcc ggt gca tgc tat gtg ccg atc gat 33
Phe Lys Ala Gly Ala Cys Tyr Val Pro Ile Asp
1 5 10

<210> 32
<211> 11
<212> PRT
<213> Xanthomonas albilineans

<400> 32

Phe Lys Ala Gly Ala Cys Tyr Val Pro Ile Asp
1 5 10

<210> 33
<211> 48
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(48)
<223> Adenylation domain subdomain III

<400> 33
ctg gcg tgc gtg atg gtg acc tcc ggc tcg acc ggc cgg ccc aag ggc 48
Leu Ala Cys Val Met Val Thr Ser Gly Ser Thr Gly Arg Pro Lys Gly
1 5 10 15

<210> 34
<211> 16
<212> PRT
<213> Xanthomonas albilineans

<400> 34

Leu Ala Cys Val Met Val Thr Ser Gly Ser Thr Gly Arg Pro Lys Gly
1 5 10 15

<210> 35
<211> 12
<212> DNA
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<220>

<221> CDS
<222> (1)..(12)
<223> Adenylation domain subdomain IV

<400> 35
ttt gcg gtg tcg 12
Phe Ala Val Ser
1

<210> 36
<211> 4
<212> PRT
<213> Xanthomonas albilineans

<400> 36

Phe Ala Val Ser
1

<210> 37
<211> 21
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(21)
<223> Adenylation domain subdomain V

<400> 37
aac aac tat ggc tgc acg gaa 21
Asn Asn Tyr Gly Cys Thr Glu
1 5

<210> 38
<211> 7
<212> PRT
<213> Xanthomonas albilineans

<400> 38

Asn Asn Tyr Gly Cys Thr Glu
1 5

<210> 39
<211> 45
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(45)
<223> Adenylation domain subdomain VI

<400> 39
ggc gag ctg cac gta cac agc gtg ggg atg gcg cgc ggc tac tgg 45

Gly Glu Leu His Val His Ser Val Gly Met Ala Arg Gly Tyr Trp
1 5 10 15

<210> 40
<211> 15
<212> PRT
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<400> 40

Gly Glu Leu His Val His Ser Val Gly Met Ala Arg Gly Tyr Trp
1 5 10 15

<210> 41
<211> 18
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(18)
<223> Adenylation domain subdomain VII

<400> 41
tac aag acc ggt gac atg 18
Tyr Lys Thr Gly Asp Met
1 5

<210> 42
<211> 6
<212> PRT
<213> Xanthomonas albilineans

<400> 42

Tyr Lys Thr Gly Asp Met
1 5

<210> 43
<211> 60
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(60)
<223> Adenylation domain subdomain VIII

<400> 43
ggc cga cag gac ttc gag gtc aag gtg cgc ggc cac cgg gtg gat acg 48
Gly Arg Gln Asp Phe Glu Val Lys Val Arg Gly His Arg Val Asp Thr
1 5 10 15

cgg cag gtg gag 60
Arg Gln Val Glu
20

<210> 44
<211> 20
<212> PRT
<213> Xanthomonas albilineans

<400> 44

Gly Arg Gln Asp Phe Glu Val Lys Val Arg Gly His Arg Val Asp Thr
1 5 10 15

Arg Gln Val Glu
20

<210> 45
<211> 21
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(21)
<223> Adenylation domain subdomain IX

<400> 45
atc gcg cac ccg tat agc gag 21
Ile Ala His Pro Tyr Ser Glu
1 5

<210> 46
<211> 7
<212> PRT
<213> Xanthomonas albilineans

<400> 46

Ile Ala His Pro Tyr Ser Glu
1 5

<210> 47
<211> 18
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(18)
<223> Adenylation domain subdomain X

<400> 47
aac ggc aag ttg gac cgg 18
Asn Gly Lys Leu Asp Arg
1 5

<210> 48
<211> 6

<212> PRT
<213> Xanthomonas albilineans

<400> 48

Asn Gly Lys Leu Asp Arg
1 5

<210> 49
<211> 33
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(33)
<223> Peptidyl carrier protein 1 domain

<400> 49
atg gat ttc ttt gcc gtc ggc ggc cat tcg gtg 33
Met Asp Phe Phe Ala Val Gly Gly His Ser Val
1 5 10

<210> 50
<211> 11
<212> PRT
<213> Xanthomonas albilineans

<400> 50

Met Asp Phe Phe Ala Val Gly Gly His Ser Val
1 5 10

<210> 51
<211> 33
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(33)
<223> Peptidyl carrier proetin 2 domain

<400> 51
gac aac ttc ttc gcc ttg ggt ggg cac tcg ctg 33
Asp Asn Phe Phe Ala Leu Gly Gly His Ser Leu
1 5 10

<210> 52
<211> 11
<212> PRT
<213> Xanthomonas albilineans

<400> 52

Asp Asn Phe Phe Ala Leu Gly Gly His Ser Leu
1 5 10

<210> 53
<211> 30
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(30)
<223> Condensation domain 1 subdomain I

<400> 53
act tat gca cag gag cgc cta tgg ctc gtc 30
Thr Tyr Ala Gln Glu Arg Leu Trp Leu Val
1 5 10

<210> 54
<211> 10
<212> PRT
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<400> 54

Thr Tyr Ala Gln Glu Arg Leu Trp Leu Val
1 5 10

<210> 55
<211> 27
<212> DNA
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<220>
<221> CDS
<222> (1)..(27)
<223> Condensation domain 1 subdomain II

<400> 55
cgg cac gaa gtg ctg cgc aca cgc ttt 27
Arg His Glu Val Leu Arg Thr Arg Phe
1 5

<210> 56
<211> 9
<212> PRT
<213> Xanthomonas albilineans

<400> 56

Arg His Glu Val Leu Arg Thr Arg Phe
1 5

<210> 57
<211> 30
<212> DNA
<213> Xanthomonas albilineans
<220>

<221> CDS
<222> (1)..(30)
<223> Condensation domain 1 subdomain III

<400> 57
atc cac cac atc att tcc gac ggc tgg tcg 30
Ile His His Ile Ile Ser Asp Gly Trp Ser
1 5 10

<210> 58
<211> 10
<212> PRT
<213> Xanthomonas albilineans

<400> 58

Ile His His Ile Ile Ser Asp Gly Trp Ser
1 5 10

<210> 59
<211> 21
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(21)
<223> Condensation domain 1 subdomain IV

<400> 59
tat gcc gac tac gcg ctg tgg 21
Tyr Ala Asp Tyr Ala Leu Trp
1 5

<210> 60
<211> 7
<212> PRT
<213> Xanthomonas albilineans

<400> 60

Tyr Ala Asp Tyr Ala Leu Trp
1 5

<210> 61
<211> 36
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(36)
<223> Condensation domain 1 subdomain V

<400> 61
atc ggc ttt ttc atc aat att ttg ccg ctg cgg gtg 36

Ile Gly Phe Phe Ile Asn Ile Leu Pro Leu Arg Val
1 5 10

<210> 62
<211> 12
<212> PRT
<213> Xanthomonas albilineans

<400> 62

Ile Gly Phe Phe Ile Asn Ile Leu Pro Leu Arg Val
1 5 10

<210> 63
<211> 21
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(21)
<223> Condensation domain 1 subdomain VI

<400> 63
gcg cac cag tcg gtg ccg ttc 21
Ala His Gln Ser Val Pro Phe
1 5

<210> 64
<211> 7
<212> PRT
<213> Xanthomonas albilineans

<400> 64

Ala His Gln Ser Val Pro Phe
1 5

<210> 65
<211> 24
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(24)
<223> Condensation domain 1 subdomain VII

<400> 65
cgc gac agt agc cag atc ccg ctg 24
Arg Asp Ser Ser Gln Ile Pro Leu
1 5

<210> 66
<211> 8
<212> PRT

<213> Xanthomonas albilineans

<400> 66

Arg Asp Ser Ser Gln Ile Pro Leu
1 5

<210> 67
<211> 30
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(30)
<223> Condensation domain 2 subdomain I

<400> 67
tcg ctg ttc cag gaa cgc ctg tgg ttc gtg 30
Ser Leu Phe Gln Glu Arg Leu Trp Phe Val
1 5 10

<210> 68
<211> 10
<212> PRT
<213> Xanthomonas albilineans

<400> 68

Ser Leu Phe Gln Glu Arg Leu Trp Phe Val
1 5 10

<210> 69
<211> 27
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(27)
<223> Condensation domain 2 subdomain II

<400> 69
cgc cac gaa atc ttg cgt acc cgc ttc 27
Arg His Glu Ile Leu Arg Thr Arg Phe
1 5

<210> 70
<211> 9
<212> PRT
<213> Xanthomonas albilineans

<400> 70

Arg His Glu Ile Leu Arg Thr Arg Phe
1 5

<210> 71
<211> 30
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(30)
<223> Condensation domain 2 subdomain III

<400> 71
atg cat cac ctt atc tac gac gct tgg tcg 30
Met His His Leu Ile Tyr Asp Ala Trp Ser
1 5 10

<210> 72
<211> 10
<212> PRT
<213> Xanthomonas albilineans

<400> 72

Met His His Leu Ile Tyr Asp Ala Trp Ser
1 5 10

<210> 73
<211> 21
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(21)
<223> Condensation domain 2 subdomain IV

<400> 73
tat gcc gac tat gcg atc tgg 21
Tyr Ala Asp Tyr Ala Ile Trp
1 5

<210> 74
<211> 7
<212> PRT
<213> Xanthomonas albilineans

<400> 74

Tyr Ala Asp Tyr Ala Ile Trp
1 5

<210> 75
<211> 33
<212> DNA
<213> Xanthomonas albilineans

<220>

<221> CDS
<222> (1)..(33)
<223> Condensation domain 2 subdomain V

<400> 75
atc ggt ttc ttc atc aac atc ttg cct ttg cgc 33
Ile Gly Phe Phe Ile Asn Ile Leu Pro Leu Arg
1 5 10

<210> 76
<211> 11
<212> PRT
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<400> 76

Ile Gly Phe Phe Ile Asn Ile Leu Pro Leu Arg
1 5 10

<210> 77
<211> 21
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(21)
<223> Condensation domain 2 subdomain VI

<400> 77
aac cag gcg cta ccg ttc gag 21
Asn Gln Ala Leu Pro Phe Glu
1 5

<210> 78
<211> 7
<212> PRT
<213> Xanthomonas albilineans

<400> 78

Asn Gln Ala Leu Pro Phe Glu
1 5

<210> 79
<211> 24
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(24)
<223> Condensation domain 2 subdomain VII

<400> 79
cgt gac acc agc cgg att ccg cta 24
Arg Asp Thr Ser Arg Ile Pro Leu

1 5

<210> 80
<211> 8
<212> PRT
<213> Xanthomonas albilineans

<400> 80

Arg Asp Thr Ser Arg Ile Pro Leu
1 5

<210> 81
<211> 242
<212> DNA
<213> Xanthomonas albilineans

<220>
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<222> (1)..(242)
<223> Bidirectional xabB promoter

<400> 81
catcacgcca cctccagcag ggtgtcatac acggccagcg gatgctgcag gttttccac: 60

ggcagggcca ctggctgtcg taagggaagc ggtgccttga gcgccggtgc ggacagtata 120

acgacacgtt ccttggccaa gcgcactgtc ggcacggcct tgctgatgcc gcccatgtag 180

ccgcgcgcct ggatctcgcg tagtagcacc acgctggccg ggatccatcg agggcgcgct 240

tg 242

<210> 82
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<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (149)..(982)
<223>

<400> 82
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ggaccgaatg cggcgggtag gcgcgactct gcgacactag cgcaatgtta tcgtcgacat 120

tgacgcccac agccctcagc gcaacgca atg ccc aat gcc gta ccg atg cag 172
Met Pro Asn Ala Val Pro Met Gln
1 5

ggc gcg cgg gga ctc ccg cag ccg caa gcg atg aac cca ggg ttg ccg 220
Gly Ala Arg Gly Leu Pro Gln Pro Gln Ala Met Asn Pro Gly Leu Pro
10 15 20

agc gtc ggc ggc ttg agc gca ggc cag cca ttg cag ttg tcg tta gca 268
Ser Val Gly Gly Leu Ser Ala Gly Gln Pro Leu Gln Leu Ser Leu Ala
25 30 35 40

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ccg gaa ctg cag gca gcc gcg cgc agt gcc cac cgc cat ctg ctc gac      316
Pro Glu Leu Gln Ala Ala Ala Arg Ser Ala His Arg His Leu Leu Asp
                45                  50                  55

gac ggc acg gcg ctt tac ctg ctg gcg ttc gat acc gcg caa ttc gac      364
Asp Gly Thr Ala Leu Tyr Leu Leu Ala Phe Asp Thr Ala Gln Phe Asp
            60                  65                  70

ccg ggg gct ttc gcg gca atg gca atc gcc cgc ccg gac agc atc gcc      412
Pro Gly Ala Phe Ala Ala Met Ala Ile Ala Arg Pro Asp Ser Ile Ala
        75                  80                  85

cgc agc gtg cgc aag cgt cag gcc gag ttc ctg ttc ggc cgt ctg gcc      460
Arg Ser Val Arg Lys Arg Gln Ala Glu Phe Leu Phe Gly Arg Leu Ala
    90                  95                  100

gcg cga ctg gcg ctg caa gag gtg ctg gga cct gcg caa gcg cag gca      508
Ala Arg Leu Ala Leu Gln Glu Val Leu Gly Pro Ala Gln Ala Gln Ala
105                 110                 115                 120

gat att gca atc ggc gcg acg cgc gcg ccc tgc tgg cct gcc ggc agc      556
Asp Ile Ala Ile Gly Ala Thr Arg Ala Pro Cys Trp Pro Ala Gly Ser
                125                 130                 135

ctg ggc agc att tcc cat tgc gag gac tac gcg gcc gcc atc gcc atg      604
Leu Gly Ser Ile Ser His Cys Glu Asp Tyr Ala Ala Ala Ile Ala Met
            140                 145                 150

gcg gcc ggc acc cgc cac ggc gtg ggc atc gat ctg gaa cga cca atc      652
Ala Ala Gly Thr Arg His Gly Val Gly Ile Asp Leu Glu Arg Pro Ile
        155                 160                 165

aca ccc gcg gcg cgc gcg gcg ttg ctg agc atc gca atc gat gcc gac      700
Thr Pro Ala Ala Arg Ala Ala Leu Leu Ser Ile Ala Ile Asp Ala Asp
    170                 175                 180

gaa gcc gct cgt ctg gca aag gcg gca gac gcg cag tgg ccg caa gac      748
Glu Ala Ala Arg Leu Ala Lys Ala Ala Asp Ala Gln Trp Pro Gln Asp
185                 190                 195                 200

ctg ctg ctg acc gca cta ttt tcg gcc aag gaa agc ctg ttc aaa gcc      796
Leu Leu Leu Thr Ala Leu Phe Ser Ala Lys Glu Ser Leu Phe Lys Ala
                205                 210                 215

gcc tac agc gcg gtc gga cgc tac ttc gac ttc agc gcg gca cgc ctg      844
Ala Tyr Ser Ala Val Gly Arg Tyr Phe Asp Phe Ser Ala Ala Arg Leu
            220                 225                 230

tgc ggc atc gac ctg gca cgg caa tgc ctg cat ctg cgc ctg acc gag      892
Cys Gly Ile Asp Leu Ala Arg Gln Cys Leu His Leu Arg Leu Thr Glu
        235                 240                 245

aca ctc tgc gcg caa ttc gtg gcc ggg caa gtg tgc gag gtc ggc ttc      940
Thr Leu Cys Ala Gln Phe Val Ala Gly Gln Val Cys Glu Val Gly Phe
    250                 255                 260

gcg cgc cta cca ccg gac ctg gtg ctc acc cac tac gcc tgg               982
Ala Arg Leu Pro Pro Asp Leu Val Leu Thr His Tyr Ala Trp
265                 270                 275

tgagcacgcg gacagtcgaa cccgccaacg ccaacggcac tcaagacgtg gcgtgcgccg    1042

cgtcggtcgt gaagctctcc ccgcagccgc actcggcggt ggcattggga ttgcggaaca    1102
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cgaaggtctc acccaagccc tgcttggcga agtcgatttc ggtgccatcg accaactgca 1162

gactggcggc atcgacataa atccgcactc cgtcctgc 1200

<210> 83
<211> 278
<212> PRT
<213> Xanthomonas albilineans

<400> 83

Met Pro Asn Ala Val Pro Met Gln Gly Ala Arg Gly Leu Pro Gln Pro
1 5 10 15

Gln Ala Met Asn Pro Gly Leu Pro Ser Val Gly Gly Leu Ser Ala Gly
20 25 30

Gln Pro Leu Gln Leu Ser Leu Ala Pro Glu Leu Gln Ala Ala Ala Arg
35 40 45

Ser Ala His Arg His Leu Leu Asp Asp Gly Thr Ala Leu Tyr Leu Leu
50 55 60

Ala Phe Asp Thr Ala Gln Phe Asp Pro Gly Ala Phe Ala Ala Met Ala
65 70 75 80

Ile Ala Arg Pro Asp Ser Ile Ala Arg Ser Val Arg Lys Arg Gln Ala
85 90 95

Glu Phe Leu Phe Gly Arg Leu Ala Ala Arg Leu Ala Leu Gln Glu Val
100 105 110

Leu Gly Pro Ala Gln Ala Gln Ala Asp Ile Ala Ile Gly Ala Thr Arg
115 120 125

Ala Pro Cys Trp Pro Ala Gly Ser Leu Gly Ser Ile Ser His Cys Glu
130 135 140

Asp Tyr Ala Ala Ala Ile Ala Met Ala Ala Gly Thr Arg His Gly Val
145 150 155 160

Gly Ile Asp Leu Glu Arg Pro Ile Thr Pro Ala Ala Arg Ala Ala Leu
165 170 175

Leu Ser Ile Ala Ile Asp Ala Asp Glu Ala Ala Arg Leu Ala Lys Ala
180 185 190

Ala Asp Ala Gln Trp Pro Gln Asp Leu Leu Leu Thr Ala Leu Phe Ser
195 200 205

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Ala Lys Glu Ser Leu Phe Lys Ala Ala Tyr Ser Ala Val Gly Arg Tyr
    210                 215                 220

Phe Asp Phe Ser Ala Ala Arg Leu Cys Gly Ile Asp Leu Ala Arg Gln
225                 230                 235                 240

Cys Leu His Leu Arg Leu Thr Glu Thr Leu Cys Ala Gln Phe Val Ala
                245                 250                 255

Gly Gln Val Cys Glu Val Gly Phe Ala Arg Leu Pro Pro Asp Leu Val
            260                 265                 270

Leu Thr His Tyr Ala Trp
        275
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<210> 84
<211> 837
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(837)
<223>

<400> 84

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Met Pro Asn Ala Val Pro Met Gln Gly Ala Arg Gly Leu Pro Gln Pro
1               5                   10                  15

caa gcg atg aac cca ggg ttg ccg agc gtc ggc ggc ttg agc gca ggc      96
Gln Ala Met Asn Pro Gly Leu Pro Ser Val Gly Gly Leu Ser Ala Gly
            20                  25                  30

cag cca ttg cag ttg tcg tta gca ccg gaa ctg cag gca gcc gcg cgc     144
Gln Pro Leu Gln Leu Ser Leu Ala Pro Glu Leu Gln Ala Ala Ala Arg
        35                  40                  45

agt gcc cac cgc cat ctg ctc gac gac ggc acg gcg ctt tac ctg ctg     192
Ser Ala His Arg His Leu Leu Asp Asp Gly Thr Ala Leu Tyr Leu Leu
    50                  55                  60

gcg ttc gat acc gcg caa ttc gac ccg ggg gct ttc gcg gca atg gca     240
Ala Phe Asp Thr Ala Gln Phe Asp Pro Gly Ala Phe Ala Ala Met Ala
65                  70                  75                  80

atc gcc cgc ccg gac agc atc gcc cgc agc gtg cgc aag cgt cag gcc     288
Ile Ala Arg Pro Asp Ser Ile Ala Arg Ser Val Arg Lys Arg Gln Ala
                85                  90                  95

gag ttc ctg ttc ggc cgt ctg gcc gcg cga ctg gcg ctg caa gag gtg     336
Glu Phe Leu Phe Gly Arg Leu Ala Ala Arg Leu Ala Leu Gln Glu Val
            100                 105                 110

ctg gga cct gcg caa gcg cag gca gat att gca atc ggc gcg acg cgc     384
Leu Gly Pro Ala Gln Ala Gln Ala Asp Ile Ala Ile Gly Ala Thr Arg
        115                 120                 125
```

```
gcg ccc tgc tgg cct gcc ggc agc ctg ggc agc att tcc cat tgc gag      432
Ala Pro Cys Trp Pro Ala Gly Ser Leu Gly Ser Ile Ser His Cys Glu
    130                 135                 140

gac tac gcg gcc gcc atc gcc atg gcg gcc ggc acc cgc cac ggc gtg      480
Asp Tyr Ala Ala Ala Ile Ala Met Ala Ala Gly Thr Arg His Gly Val
145                 150                 155                 160

ggc atc gat ctg gaa cga cca atc aca ccc gcg gcg cgc gcg gcg ttg      528
Gly Ile Asp Leu Glu Arg Pro Ile Thr Pro Ala Ala Arg Ala Ala Leu
                165                 170                 175

ctg agc atc gca atc gat gcc gac gaa gcc gct cgt ctg gca aag gcg      576
Leu Ser Ile Ala Ile Asp Ala Asp Glu Ala Ala Arg Leu Ala Lys Ala
            180                 185                 190

gca gac gcg cag tgg ccg caa gac ctg ctg ctg acc gca cta ttt tcg      624
Ala Asp Ala Gln Trp Pro Gln Asp Leu Leu Leu Thr Ala Leu Phe Ser
        195                 200                 205

gcc aag gaa agc ctg ttc aaa gcc gcc tac agc gcg gtc gga cgc tac      672
Ala Lys Glu Ser Leu Phe Lys Ala Ala Tyr Ser Ala Val Gly Arg Tyr
    210                 215                 220

ttc gac ttc agc gcg gca cgc ctg tgc ggc atc gac ctg gca cgg caa      720
Phe Asp Phe Ser Ala Ala Arg Leu Cys Gly Ile Asp Leu Ala Arg Gln
225                 230                 235                 240
tgc ctg cat ctg cgc ctg acc gag aca ctc tgc gcg caa ttc gtg gcc      768
Cys Leu His Leu Arg Leu Thr Glu Thr Leu Cys Ala Gln Phe Val Ala
                245                 250                 255

ggg caa gtg tgc gag gtc ggc ttc gcg cgc cta cca ccg gac ctg gtg      816
Gly Gln Val Cys Glu Val Gly Phe Ala Arg Leu Pro Pro Asp Leu Val
            260                 265                 270

ctc acc cac tac gcc tgg tga                                         837
Leu Thr His Tyr Ala Trp
        275
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<210> 85
<211> 278
<212> PRT
<213> Xanthomonas albilineans

<400> 85

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Met Pro Asn Ala Val Pro Met Gln Gly Ala Arg Gly Leu Pro Gln Pro
1               5                   10                  15

Gln Ala Met Asn Pro Gly Leu Pro Ser Val Gly Gly Leu Ser Ala Gly
            20                  25                  30

Gln Pro Leu Gln Leu Ser Leu Ala Pro Glu Leu Gln Ala Ala Ala Arg
        35                  40                  45

Ser Ala His Arg His Leu Leu Asp Asp Gly Thr Ala Leu Tyr Leu Leu
    50                  55                  60
```

```
Ala Phe Asp Thr Ala Gln Phe Asp Pro Gly Ala Phe Ala Ala Met Ala
65                  70                  75                  80

Ile Ala Arg Pro Asp Ser Ile Ala Arg Ser Val Arg Lys Arg Gln Ala
                85                  90                  95

Glu Phe Leu Phe Gly Arg Leu Ala Ala Arg Leu Ala Leu Gln Glu Val
            100                 105                 110

Leu Gly Pro Ala Gln Ala Gln Ala Asp Ile Ala Ile Gly Ala Thr Arg
        115                 120                 125

Ala Pro Cys Trp Pro Ala Gly Ser Leu Gly Ser Ile Ser His Cys Glu
    130                 135                 140

Asp Tyr Ala Ala Ala Ile Ala Met Ala Ala Gly Thr Arg His Gly Val
145                 150                 155                 160

Gly Ile Asp Leu Glu Arg Pro Ile Thr Pro Ala Ala Arg Ala Ala Leu
                165                 170                 175

Leu Ser Ile Ala Ile Asp Ala Asp Glu Ala Ala Arg Leu Ala Lys Ala
            180                 185                 190

Ala Asp Ala Gln Trp Pro Gln Asp Leu Leu Leu Thr Ala Leu Phe Ser
        195                 200                 205

Ala Lys Glu Ser Leu Phe Lys Ala Ala Tyr Ser Ala Val Gly Arg Tyr
    210                 215                 220

Asp Phe Ser Ala Ala Arg Leu Cys Gly Ile Asp Leu Ala Arg Gln
225                 230                 235                 240

Cys Leu His Leu Arg Leu Thr Glu Thr Leu Cys Ala Gln Phe Val Ala
                245                 250                 255

Gly Gln Val Cys Glu Val Gly Phe Ala Arg Leu Pro Pro Asp Leu Val
            260                 265                 270

Leu Thr His Tyr Ala Trp
        275
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<210> 86
<211> 180
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS

<222> (1)..(168)
<223>

<400> 86
ggc gtg ggc atc gat ctg gaa cga cca atc aca ccc gcg gcg cgc gcg 48
Gly Val Gly Ile Asp Leu Glu Arg Pro Ile Thr Pro Ala Ala Arg Ala
1 5 10 15

gcg ttg ctg agc atc gca atc gat gcc gac gaa gcc gct cgt ctg gca 96
Ala Leu Leu Ser Ile Ala Ile Asp Ala Asp Glu Ala Ala Arg Leu Ala
20 25 30

aag gcg gca gac gcg cag tgg ccg caa gac ctg ctg ctg acc gca cta 144
Lys Ala Ala Asp Ala Gln Trp Pro Gln Asp Leu Leu Leu Thr Ala Leu
35 40 45

ttt tcg gcc aag gaa agc ctg ttc aaagccgcct ac 180
Phe Ser Ala Lys Glu Ser Leu Phe
50 55

<210> 87
<211> 56
<212> PRT
<213> Xanthomonas albilineans

<400> 87

Gly Val Gly Ile Asp Leu Glu Arg Pro Ile Thr Pro Ala Ala Arg Ala
1 5 10 15

Ala Leu Leu Ser Ile Ala Ile Asp Ala Asp Glu Ala Ala Arg Leu Ala
20 25 30

Lys Ala Ala Asp Ala Gln Trp Pro Gln Asp Leu Leu Leu Thr Ala Leu
35 40 45

Phe Ser Ala Lys Glu Ser Leu Phe
50 55

<210> 88
<211> 27
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(27)
<223>

<400> 88
ggc gtg ggc atc gat ctg gaa cga cca 27
Gly Val Gly Ile Asp Leu Glu Arg Pro
1 5

<210> 89

<211> 9
<212> PRT
<213> Xanthomonas albilineans

<400> 89

Gly Val Gly Ile Asp Leu Glu Arg Pro
1 5

<210> 90
<211> 117
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(117)
<223>

<400> 90
atc aca ccc gcg gcg cgc gcg gcg ttg ctg agc atc gca atc gat gcc 48
Ile Thr Pro Ala Ala Arg Ala Ala Leu Leu Ser Ile Ala Ile Asp Ala
1 5 10 15

gac gaa gcc gct cgt ctg gca aag gcg gca gac gcg cag tgg ccg caa 96
Asp Glu Ala Ala Arg Leu Ala Lys Ala Ala Asp Ala Gln Trp Pro Gln
20 25 30

gac ctg ctg ctg acc gca cta 117
Asp Leu Leu Leu Thr Ala Leu
35

<210> 91
<211> 39
<212> PRT
<213> Xanthomonas albilineans

<400> 91

Ile Thr Pro Ala Ala Arg Ala Ala Leu Leu Ser Ile Ala Ile Asp Ala
1 5 10 15

Asp Glu Ala Ala Arg Leu Ala Lys Ala Ala Asp Ala Gln Trp Pro Gln
20 25 30

Asp Leu Leu Leu Thr Ala Leu
35

<210> 92
<211> 36
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(36)
<223>

<400> 92
ttt tcg gcc aag gaa agc ctg ttc aaa gcc gcc tac 36
Phe Ser Ala Lys Glu Ser Leu Phe Lys Ala Ala Tyr
1 5 10

<210> 93
<211> 12
<212> PRT
<213> Xanthomonas albilineans

<400> 93

Phe Ser Ala Lys Glu Ser Leu Phe Lys Ala Ala Tyr
1 5 10

<210> 94
<211> 1515
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (46)..(1071)
<223>

<400> 94
caaaagccgg ccgccgtcac ccgttcatcg atagcgaggg caatc atg gat tca gcg 57
Met Asp Ser Ala
1

tta cct aca tct gca ttt acc ttc gat ctc ttt tac acc acg gtt aac 105
Leu Pro Thr Ser Ala Phe Thr Phe Asp Leu Phe Tyr Thr Thr Val Asn
5 10 15 20

gcc tac tat cgc act gcc gca gtc aag gcg gcg atc gaa ctg ggg cta 153
Ala Tyr Tyr Arg Thr Ala Ala Val Lys Ala Ala Ile Glu Leu Gly Leu
25 30 35

ttc gat gtg gtg ggg cag cag ggc cga act ccc gca gcc atc gcc gag 201
Phe Asp Val Val Gly Gln Gln Gly Arg Thr Pro Ala Ala Ile Ala Glu
40 45 50

gcc tgc cag gcg tcg ccg cgc ggc att cgc atc ctt tgc tat tac cta 249
Ala Cys Gln Ala Ser Pro Arg Gly Ile Arg Ile Leu Cys Tyr Tyr Leu
55 60 65

gta tcg atc ggt ttt cta cgc cgc aac ggt ggc ctg ttc tac ata gat 297
Val Ser Ile Gly Phe Leu Arg Arg Asn Gly Gly Leu Phe Tyr Ile Asp
70 75 80

cgc aac atg gcc atg tac ctg gat cgt agt tcg ccc ggc tac ctg ggt 345
Arg Asn Met Ala Met Tyr Leu Asp Arg Ser Ser Pro Gly Tyr Leu Gly
85 90 95 100

ggc agc atc aag ttc ctg ctc tcg ccc tac atc atg agc gcc ttc acc 393
Gly Ser Ile Lys Phe Leu Leu Ser Pro Tyr Ile Met Ser Ala Phe Thr
105 110 115

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gat ctg acc gcc gta gtc agg acc ggc aag atc aac ctg gcg cag gac      441
Asp Leu Thr Ala Val Val Arg Thr Gly Lys Ile Asn Leu Ala Gln Asp
            120                 125                 130

ggc gtg gtg gca ccg gat cac ccg cag tgg gtg gaa ttt gca cgc gcg      489
Gly Val Val Ala Pro Asp His Pro Gln Trp Val Glu Phe Ala Arg Ala
        135                 140                 145

atg gca ccg atg atg gcg ctg ccc tcg gcg ttg atc gcc aat atg gtg      537
Met Ala Pro Met Met Ala Leu Pro Ser Ala Leu Ile Ala Asn Met Val
    150                 155                 160

tcg ttg ccc gct gat cgg ccg att cgt gtg ctg gac gtg gca gcc ggc      585
Ser Leu Pro Ala Asp Arg Pro Ile Arg Val Leu Asp Val Ala Ala Gly
165                 170                 175                 180

cac ggc ctg ttc ggc atc gcc ttc gcg cag cgc ttc cgc cag gct gaa      633
His Gly Leu Phe Gly Ile Ala Phe Ala Gln Arg Phe Arg Gln Ala Glu
                185                 190                 195

gtg agc ttc ctg gac tgg gac aac gtg cta gac gta gca cgc gaa aac      681
Val Ser Phe Leu Asp Trp Asp Asn Val Leu Asp Val Ala Arg Glu Asn
            200                 205                 210

gcc cag gcg gcc aaa gtg gcc gag cga gcg cgt ttc ctg ccc ggc aac      729
Ala Gln Ala Ala Lys Val Ala Glu Arg Ala Arg Phe Leu Pro Gly Asn
        215                 220                 225

gca ttc gac ctc gat tac ggc agc ggc tac gac gtg atc ttg ttg acc      777
Ala Phe Asp Leu Asp Tyr Gly Ser Gly Tyr Asp Val Ile Leu Leu Thr
    230                 235                 240

aac ttc ctg cac cat ttc gat gag gtc gat ggc gag cgc atc ttg gct      825
Asn Phe Leu His His Phe Asp Glu Val Asp Gly Glu Arg Ile Leu Ala
245                 250                 255                 260

aag acg cgc gat gcg ctg aac gac gac ggc atg gtg atc act ttc gaa      873
Lys Thr Arg Asp Ala Leu Asn Asp Asp Gly Met Val Ile Thr Phe Glu
                265                 270                 275

ttc atc gcc gac gaa gag cgt tcc tca ccg ccg ctg gcc gcc acc ttc      921
Phe Ile Ala Asp Glu Glu Arg Ser Ser Pro Pro Leu Ala Ala Thr Phe
            280                 285                 290

agc atg atg atg ctg ggc acc acc ccg gcg ggc gag tcc tac acc tat      969
Ser Met Met Met Leu Gly Thr Thr Pro Ala Gly Glu Ser Tyr Thr Tyr
        295                 300                 305

agc gat ctg gaa agg atg ttt cgg cat gcc ggc ttc ggc cac gtg gaa     1017
Ser Asp Leu Glu Arg Met Phe Arg His Ala Gly Phe Gly His Val Glu
    310                 315                 320

cta aaa tcg ata ccg ccg gcc ttg ctg aaa gtg gtg gtt tcc cgc aag     1065
Leu Lys Ser Ile Pro Pro Ala Leu Leu Lys Val Val Val Ser Arg Lys
325                 330                 335                 340

agg gcc ccataatgat cgaatcggcg acatcccctg tggcgaaaac cgagcgcatc      1121
Arg Ala

tggtgcaccg agctggacct ggatgcactc aacgccatgt cggccaacac gatgcaggcc   1181

ctgctcggta tacgcatgat cgagatcggc tcggactatc tggtctcctg catgtcggtg   1241
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gactggcgtt gccaccagcc ctatggggta ttgcatggcg gcgcatcggt caccctggcc 1301

gaggctaccg gcagcatggc ggcctccatg tgcgtgccgg ccggccaacg ttgcgttggc 1361

ctagacatca atgccaacca catcgcgagc atctccagtg gccaagtaca gtgcatcgcg 1421

cggccgctgc acataggggc cttgacccag gtatggcaga tgcgcatcta tgacgaaggt 1481

gaccgcacga tctgcgtgtc gcgcctgacc atgg 1515

<210> 95
<211> 342
<212> PRT
<213> Xanthomonas albilineans
<400> 95

Met Asp Ser Ala Leu Pro Thr Ser Ala Phe Thr Phe Asp Leu Phe Tyr
1 5 10 15

Thr Thr Val Asn Ala Tyr Tyr Arg Thr Ala Ala Val Lys Ala Ala Ile
20 25 30

Glu Leu Gly Leu Phe Asp Val Val Gly Gln Gln Gly Arg Thr Pro Ala
35 40 45

Ala Ile Ala Glu Ala Cys Gln Ala Ser Pro Arg Gly Ile Arg Ile Leu
50 55 60

Cys Tyr Tyr Leu Val Ser Ile Gly Phe Leu Arg Arg Asn Gly Gly Leu
65 70 75 80

Phe Tyr Ile Asp Arg Asn Met Ala Met Tyr Leu Asp Arg Ser Ser Pro
85 90 95

Gly Tyr Leu Gly Gly Ser Ile Lys Phe Leu Leu Ser Pro Tyr Ile Met
100 105 110

Ser Ala Phe Thr Asp Leu Thr Ala Val Val Arg Thr Gly Lys Ile Asn
115 120 125

Leu Ala Gln Asp Gly Val Val Ala Pro Asp His Pro Gln Trp Val Glu
130 135 140

Phe Ala Arg Ala Met Ala Pro Met Met Ala Leu Pro Ser Ala Leu Ile
145 150 155 160

Ala Asn Met Val Ser Leu Pro Ala Asp Arg Pro Ile Arg Val Leu Asp
165 170 175

Val Ala Ala Gly His Gly Leu Phe Gly Ile Ala Phe Ala Gln Arg Phe

180 185 190

Arg Gln Ala Glu Val Ser Phe Leu Asp Trp Asp Asn Val Leu Asp Val
195 200 205

Ala Arg Glu Asn Ala Gln Ala Ala Lys Val Ala Glu Arg Ala Arg Phe
210 215 220

Leu Pro Gly Asn Ala Phe Asp Leu Asp Tyr Gly Ser Gly Tyr Asp Val
225 230 235 240

Ile Leu Leu Thr Asn Phe Leu His His Phe Asp Glu Val Asp Gly Glu
245 250 255

Arg Ile Leu Ala Lys Thr Arg Asp Ala Leu Asn Asp Asp Gly Met Val
260 265 270

Ile Thr Phe Glu Phe Ile Ala Asp Glu Glu Arg Ser Ser Pro Pro Leu
275 280 285

Ala Ala Thr Phe Ser Met Met Met Leu Gly Thr Thr Pro Ala Gly Glu
290 295 300

Ser Tyr Thr Tyr Ser Asp Leu Glu Arg Met Phe Arg His Ala Gly Phe
305 310 315 320

Gly His Val Glu Leu Lys Ser Ile Pro Pro Ala Leu Leu Lys Val Val
325 330 335

Val Ser Arg Lys Arg Ala
340

<210> 96
<211> 1032
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(1029)
<223>

<400> 96
atg gat tca gcg tta cct aca tct gca ttt acc ttc gat ctc ttt tac 48
Met Asp Ser Ala Leu Pro Thr Ser Ala Phe Thr Phe Asp Leu Phe Tyr
1 5 10 15

acc acg gtt aac gcc tac tat cgc act gcc gca gtc aag gcg gcg atc 96
Thr Thr Val Asn Ala Tyr Tyr Arg Thr Ala Ala Val Lys Ala Ala Ile
20 25 30

gaa ctg ggg cta ttc gat gtg gtg ggg cag cag ggc cga act ccc gca 144

```
Glu Leu Gly Leu Phe Asp Val Val Gly Gln Gln Gly Arg Thr Pro Ala
        35                  40                  45

gcc atc gcc gag gcc tgc cag gcg tcg ccg cgc ggc att cgc atc ctt      192
Ala Ile Ala Glu Ala Cys Gln Ala Ser Pro Arg Gly Ile Arg Ile Leu
    50                  55                  60

tgc tat tac cta gta tcg atc ggt ttt cta cgc cgc aac ggt ggc ctg      240
Cys Tyr Tyr Leu Val Ser Ile Gly Phe Leu Arg Arg Asn Gly Gly Leu
65                  70                  75                  80

ttc tac ata gat cgc aac atg gcc atg tac ctg gat cgt agt tcg ccc      288
Phe Tyr Ile Asp Arg Asn Met Ala Met Tyr Leu Asp Arg Ser Ser Pro
                85                  90                  95

ggc tac ctg ggt ggc agc atc aag ttc ctg ctc tcg ccc tac atc atg      336
Gly Tyr Leu Gly Gly Ser Ile Lys Phe Leu Leu Ser Pro Tyr Ile Met
            100                 105                 110

agc gcc ttc acc gat ctg acc gcc gta gtc agg acc ggc aag atc aac      384
Ser Ala Phe Thr Asp Leu Thr Ala Val Val Arg Thr Gly Lys Ile Asn
        115                 120                 125

ctg gcg cag gac ggc gtg gtg gca ccg gat cac ccg cag tgg gtg gaa      432
Leu Ala Gln Asp Gly Val Val Ala Pro Asp His Pro Gln Trp Val Glu
    130                 135                 140

ttt gca cgc gcg atg gca ccg atg atg gcg ctg ccc tcg gcg ttg atc      480
Phe Ala Arg Ala Met Ala Pro Met Met Ala Leu Pro Ser Ala Leu Ile
145                 150                 155                 160

gcc aat atg gtg tcg ttg ccc gct gat cgg ccg att cgt gtg ctg gac      528
Ala Asn Met Val Ser Leu Pro Ala Asp Arg Pro Ile Arg Val Leu Asp
                165                 170                 175

gtg gca gcc ggc cac ggc ctg ttc ggc atc gcc ttc gcg cag cgc ttc      576
Val Ala Ala Gly His Gly Leu Phe Gly Ile Ala Phe Ala Gln Arg Phe
            180                 185                 190

cgc cag gct gaa gtg agc ttc ctg gac tgg gac aac gtg cta gac gta      624
Arg Gln Ala Glu Val Ser Phe Leu Asp Trp Asp Asn Val Leu Asp Val
        195                 200                 205

gca cgc gaa aac gcc cag gcg gcc aaa gtg gcc gag cga gcg cgt ttc      672
Ala Arg Glu Asn Ala Gln Ala Ala Lys Val Ala Glu Arg Ala Arg Phe
    210                 215                 220

ctg ccc ggc aac gca ttc gac ctc gat tac ggc agc ggc tac gac gtg      720
Leu Pro Gly Asn Ala Phe Asp Leu Asp Tyr Gly Ser Gly Tyr Asp Val
225                 230                 235                 240

atc ttg ttg acc aac ttc ctg cac cat ttc gat gag gtc gat ggc gag      768
Ile Leu Leu Thr Asn Phe Leu His His Phe Asp Glu Val Asp Gly Glu
                245                 250                 255

cgc atc ttg gct aag acg cgc gat gcg ctg aac gac gac ggc atg gtg      816
Arg Ile Leu Ala Lys Thr Arg Asp Ala Leu Asn Asp Asp Gly Met Val
            260                 265                 270

atc act ttc gaa ttc atc gcc gac gaa gag cgt tcc tca ccg ccg ctg      864
Ile Thr Phe Glu Phe Ile Ala Asp Glu Glu Arg Ser Ser Pro Pro Leu
        275                 280                 285
```

```
gcc gcc acc ttc agc atg atg atg ctg ggc acc acc ccg gcg ggc gag      912
Ala Ala Thr Phe Ser Met Met Met Leu Gly Thr Thr Pro Ala Gly Glu
    290                 295                 300

tcc tac acc tat agc gat ctg gaa agg atg ttt cgg cat gcc ggc ttc      960
Ser Tyr Thr Tyr Ser Asp Leu Glu Arg Met Phe Arg His Ala Gly Phe
305                 310                 315                 320

ggc cac gtg gaa cta aaa tcg ata ccg ccg gcc ttg ctg aaa gtg gtg     1008
Gly His Val Glu Leu Lys Ser Ile Pro Pro Ala Leu Leu Lys Val Val
                325                 330                 335

gtt tcc cgc aag agg gcc cca taa                                    1032
Val Ser Arg Lys Arg Ala Pro
            340
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<210> 97
<211> 343
<212> PRT
<213> Xanthomonas albilineans

<400> 97

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Met Asp Ser Ala Leu Pro Thr Ser Ala Phe Thr Phe Asp Leu Phe Tyr
1               5                   10                  15

Thr Thr Val Asn Ala Tyr Tyr Arg Thr Ala Ala Val Lys Ala Ala Ile
            20                  25                  30

Glu Leu Gly Leu Phe Asp Val Val Gly Gln Gln Gly Arg Thr Pro Ala
        35                  40                  45

Ala Ile Ala Glu Ala Cys Gln Ala Ser Pro Arg Gly Ile Arg Ile Leu
    50                  55                  60

Cys Tyr Tyr Leu Val Ser Ile Gly Phe Leu Arg Arg Asn Gly Gly Leu
65                  70                  75                  80

Phe Tyr Ile Asp Arg Asn Met Ala Met Tyr Leu Asp Arg Ser Ser Pro
                85                  90                  95

Gly Tyr Leu Gly Gly Ser Ile Lys Phe Leu Leu Ser Pro Tyr Ile Met
            100                 105                 110

Ser Ala Phe Thr Asp Leu Thr Ala Val Val Arg Thr Gly Lys Ile Asn
        115                 120                 125

Leu Ala Gln Asp Gly Val Val Ala Pro Asp His Pro Gln Trp Val Glu
    130                 135                 140

Phe Ala Arg Ala Met Ala Pro Met Met Ala Leu Pro Ser Ala Leu Ile
145                 150                 155                 160
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Ala Asn Met Val Ser Leu Pro Ala Asp Arg Pro Ile Arg Val Leu Asp
165 170 175

Val Ala Ala Gly His Gly Leu Phe Gly Ile Ala Phe Ala Gln Arg Phe
180 185 190

Arg Gln Ala Glu Val Ser Phe Leu Asp Trp Asp Asn Val Leu Asp Val
195 200 205

Ala Arg Glu Asn Ala Gln Ala Ala Lys Val Ala Glu Arg Ala Arg Phe
210 215 220

Leu Pro Gly Asn Ala Phe Asp Leu Asp Tyr Gly Ser Gly Tyr Asp Val
225 230 235 240

Ile Leu Leu Thr Asn Phe Leu His His Phe Asp Glu Val Asp Gly Glu
245 250 255

Arg Ile Leu Ala Lys Thr Arg Asp Ala Leu Asn Asp Asp Gly Met Val
260 265 270

Ile Thr Phe Glu Phe Ile Ala Asp Glu Glu Arg Ser Ser Pro Pro Leu
275 280 285

Ala Ala Thr Phe Ser Met Met Met Leu Gly Thr Thr Pro Ala Gly Glu
290 295 300

Ser Tyr Thr Tyr Ser Asp Leu Glu Arg Met Phe Arg His Ala Gly Phe
305 310 315 320

Gly His Val Glu Leu Lys Ser Ile Pro Pro Ala Leu Leu Lys Val Val
325 330 335

Val Ser Arg Lys Arg Ala Pro
340

<210> 98
<211> 21
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(21)
<223> Motif I

<400> 98
gtg ctg gac gtg gca gcc ggc 21
Val Leu Asp Val Ala Ala Gly
1 5

<210> 99
<211> 7
<212> PRT
<213> Xanthomonas albilineans
<400> 99

Val Leu Asp Val Ala Ala Gly
1 5

<210> 100
<211> 24
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(24)
<223> Motif II

<400> 100
agc ggc tac gac gtg atc ttg ttg 24
Ser Gly Tyr Asp Val Ile Leu Leu
1 5

<210> 101
<211> 8
<212> PRT
<213> Xanthomonas albilineans

<400> 101

Ser Gly Tyr Asp Val Ile Leu Leu
1 5

<210> 102
<211> 27
<212> DNA
<213> Xanthomonas albilineans

<220>
<221> CDS
<222> (1)..(27)
<223> Motif III

<400> 102
ctg aac gac gac ggc atg gtg atc act 27
Leu Asn Asp Asp Gly Met Val Ile Thr
1 5

<210> 103
<211> 9
<212> PRT
<213> Xanthomonas albilineans

<400> 103

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Leu Asn Asp Asp Gly Met Val Ile Thr
1               5

<210>  104
<211>  303
<212>  DNA
<213>  Xanthomonas albilineans

<220>
<221>  CDS
<222>  (1)..(303)
<223>


<400>  104
gtg ctg gac gtg gca gcc ggc cac ggc ctg ttc ggc atc gcc ttc gcg        48
Val Leu Asp Val Ala Ala Gly His Gly Leu Phe Gly Ile Ala Phe Ala
1               5                   10                  15

cag cgc ttc cgc cag gct gaa gtg agc ttc ctg gac tgg gac aac gtg        96
Gln Arg Phe Arg Gln Ala Glu Val Ser Phe Leu Asp Trp Asp Asn Val
            20                  25                  30

cta gac gta gca cgc gaa aac gcc cag gcg gcc aaa gtg gcc gag cga       144
Leu Asp Val Ala Arg Glu Asn Ala Gln Ala Ala Lys Val Ala Glu Arg
        35                  40                  45

gcg cgt ttc ctg ccc ggc aac gca ttc gac ctc gat tac ggc agc ggc       192
Ala Arg Phe Leu Pro Gly Asn Ala Phe Asp Leu Asp Tyr Gly Ser Gly
    50                  55                  60

tac gac gtg atc ttg ttg acc aac ttc ctg cac cat ttc gat gag gtc       240
Tyr Asp Val Ile Leu Leu Thr Asn Phe Leu His His Phe Asp Glu Val
65                  70                  75                  80

gat ggc gag cgc atc ttg gct aag acg cgc gat gcg ctg aac gac gac       288
Asp Gly Glu Arg Ile Leu Ala Lys Thr Arg Asp Ala Leu Asn Asp Asp
                85                  90                  95

ggc atg gtg atc act                                                  303
Gly Met Val Ile Thr
            100


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<211>  101
<212>  PRT
<213>  Xanthomonas albilineans

<400>  105

Val Leu Asp Val Ala Ala Gly His Gly Leu Phe Gly Ile Ala Phe Ala
1               5                   10                  15

Gln Arg Phe Arg Gln Ala Glu Val Ser Phe Leu Asp Trp Asp Asn Val
            20                  25                  30

Leu Asp Val Ala Arg Glu Asn Ala Gln Ala Ala Lys Val Ala Glu Arg
        35                  40                  45
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Ala Arg Phe Leu Pro Gly Asn Ala Phe Asp Leu Asp Tyr Gly Ser Gly
50 55 60

Tyr Asp Val Ile Leu Leu Thr Asn Phe Leu His His Phe Asp Glu Val
65 70 75 80

Asp Gly Glu Arg Ile Leu Ala Lys Thr Arg Asp Ala Leu Asn Asp Asp
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Gly Met Val Ile Thr
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1 5 10 15

acc acg gtt aac gcc tac tat cgc act gcc gca gtc aag gcg gcg atc 96
Thr Thr Val Asn Ala Tyr Tyr Arg Thr Ala Ala Val Lys Ala Ala Ile
20 25 30

gaa ctg ggg cta ttc gat gtg gtg ggg cag cag ggc cga act ccc gca 144
Glu Leu Gly Leu Phe Asp Val Val Gly Gln Gln Gly Arg Thr Pro Ala
35 40 45

gcc atc gcc gag gcc tgc cag gcg tcg ccg cgc ggc att cgc atc ctt 192
Ala Ile Ala Glu Ala Cys Gln Ala Ser Pro Arg Gly Ile Arg Ile Leu
50 55 60

tgc tat tac cta gta tcg atc ggt ttt cta cgc cgc aac ggt ggc ctg 240
Cys Tyr Tyr Leu Val Ser Ile Gly Phe Leu Arg Arg Asn Gly Gly Leu
65 70 75 80

ttc tac ata gat cgc aac atg gcc atg tac ctg gat cgt agt tcg ccc 288
Phe Tyr Ile Asp Arg Asn Met Ala Met Tyr Leu Asp Arg Ser Ser Pro
85 90 95

ggc tac ctg ggt ggc agc atc aag ttc ctg ctc tcg ccc tac atc atg 336
Gly Tyr Leu Gly Gly Ser Ile Lys Phe Leu Leu Ser Pro Tyr Ile Met
100 105 110

agc gcc ttc acc gat ctg acc gcc gta gtc agg acc ggc aag atc aac 384
Ser Ala Phe Thr Asp Leu Thr Ala Val Val Arg Thr Gly Lys Ile Asn
115 120 125

ctg gcg cag gac ggc gtg gtg gca ccg gat cac ccg cag tgg gtg gaa 432
Leu Ala Gln Asp Gly Val Val Ala Pro Asp His Pro Gln Trp Val Glu

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    130                 135                 140

ttt gca cgc gcg atg gca ccg atg atg gcg ctg ccc tcg gcg ttg atc      480
Phe Ala Arg Ala Met Ala Pro Met Met Ala Leu Pro Ser Ala Leu Ile
145                 150                 155                 160

gcc aat atg gtg tcg ttg ccc gct gat cgg ccg att cgt gtg ctg gac      528
Ala Asn Met Val Ser Leu Pro Ala Asp Arg Pro Ile Arg Val Leu Asp
                165                 170                 175

gtg gca gcc ggc cac ggc ctg ttc ggc atc gcc ttc gcg cag cgc ttc      576
Val Ala Ala Gly His Gly Leu Phe Gly Ile Ala Phe Ala Gln Arg Phe
            180                 185                 190

cgc cag gct gaa gtg agc ttc ctg gac tgg gac aac gtg cta gac gta      624
Arg Gln Ala Glu Val Ser Phe Leu Asp Trp Asp Asn Val Leu Asp Val
        195                 200                 205

gca cgc gaa aac gcc cag gcg gcc aaa gtg gcc gag cga gcg cgt ttc      672
Ala Arg Glu Asn Ala Gln Ala Ala Lys Val Ala Glu Arg Ala Arg Phe
    210                 215                 220

ctg ccc ggc aac gca ttc gac ctc gat tac ggc agc ggc tac gac gtg      720
Leu Pro Gly Asn Ala Phe Asp Leu Asp Tyr Gly Ser Gly Tyr Asp Val
225                 230                 235                 240

atc ttg ttg acc aac ttc ctg cac cat ttc gat gag gtc gat ggc gag      768
Ile Leu Leu Thr Asn Phe Leu His His Phe Asp Glu Val Asp Gly Glu
                245                 250                 255

cgc atc ttg gct aag acg cgc gat gcg ctg aac gac gac ggc atg gtg      816
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            260                 265                 270

atc act ttc gaa ttc                                                 831
Ile Thr Phe Glu Phe
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<211> 277
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<213> Xanthomonas albilineans

<400> 107

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1               5                   10                  15

Thr Thr Val Asn Ala Tyr Tyr Arg Thr Ala Ala Val Lys Ala Ala Ile
            20                  25                  30

Glu Leu Gly Leu Phe Asp Val Val Gly Gln Gln Gly Arg Thr Pro Ala
        35                  40                  45

Ala Ile Ala Glu Ala Cys Gln Ala Ser Pro Arg Gly Ile Arg Ile Leu
    50                  55                  60

Cys Tyr Tyr Leu Val Ser Ile Gly Phe Leu Arg Arg Asn Gly Gly Leu
65                  70                  75                  80
```

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Phe Tyr Ile Asp Arg Asn Met Ala Met Tyr Leu Asp Arg Ser Ser Pro
                85                  90                  95

Gly Tyr Leu Gly Gly Ser Ile Lys Phe Leu Leu Ser Pro Tyr Ile Met
            100                 105                 110

Ser Ala Phe Thr Asp Leu Thr Ala Val Val Arg Thr Gly Lys Ile Asn
        115                 120                 125

Leu Ala Gln Asp Gly Val Val Ala Pro Asp His Pro Gln Trp Val Glu
    130                 135                 140

Phe Ala Arg Ala Met Ala Pro Met Met Ala Leu Pro Ser Ala Leu Ile
145                 150                 155                 160

Ala Asn Met Val Ser Leu Pro Ala Asp Arg Pro Ile Arg Val Leu Asp
                165                 170                 175

Val Ala Ala Gly His Gly Leu Phe Gly Ile Ala Phe Ala Gln Arg Phe
            180                 185                 190

Arg Gln Ala Glu Val Ser Phe Leu Asp Trp Asp Asn Val Leu Asp Val
        195                 200                 205

Ala Arg Glu Asn Ala Gln Ala Ala Lys Val Ala Glu Arg Ala Arg Phe
    210                 215                 220

Leu Pro Gly Asn Ala Phe Asp Leu Asp Tyr Gly Ser Gly Tyr Asp Val
225                 230                 235                 240

Ile Leu Leu Thr Asn Phe Leu His His Phe Asp Glu Val Asp Gly Glu
                245                 250                 255

Arg Ile Leu Ala Lys Thr Arg Asp Ala Leu Asn Asp Asp Gly Met Val
            260                 265                 270

Ile Thr Phe Glu Phe
        275
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/01190

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C07K 14/195; C07H 21/04; C12N 15/52, 15/62

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN: File Reg, File CA (subsequence search of the individual sequences of Claim 1 combined with keywords ketoacyl reductase, polyketide, antibiotic, xanthomonas, albicidin in File CA), Index (CA, WPI, Medline, keyword xanthomonas albilineans, polyketide, gene)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STN FILE MEDLINE ABSTRACT 2001060291 & G. HUANG *et al.*, Gene, 255(2), September 19 2000, pp. 327-333. See abstract and GENBANK sequences AF239749 and AF238750 and CAS Registry number 332004-68-9.	1-94
X	PUBMED ABSTRACT 10780924 & F. SCHAUWECKER *et al.*, Chem. Biol., April 2000, 7(4), pp. 287-297. See abstract and GenPept sequence AAF42473, positions 947-952	1-94
P,X	STN FILE MEDLINE ABSTRACT 20011526256 & G. HUANG *et al.*, Microbiology, March 2001, 147(3), pp. 631-642. See abstract.	1-94

☒ Further documents are listed in the continuation of Box C ☐ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
28 November 2001

Date of mailing of the international search report
18 DEC 2001

Name and mailing address of the ISA/AU

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/01190

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	STN FILE MEDLINE ABSTRACT 2001087312 & G. HUANG *et al.*, Gene, 258(1-2), November 27 2000, pp. 193-199. See abstract and CAS Registry number 346735-79-3.	1-94
P,X	STN FILE MEDLINE ABSTRACT 2001122130 & G. HUANG *et al.*, FEMS Microbiology Letters, December 1 2000, pp. 129-136. See abstract.	1-94
X	STN FILE CA-ABSTRACT 132:103595 & K. MAYER et al., Nature, 1999, 402(6763), pp. 769-777. See abstract and CAS Registry number 254869-45-9	1-94
X	STN FILE CA ABSTRACT 130:120325 & S. T. COLE et al., Nature 1998, 396(6707), pp. 190-198. See abstract and CAS Registry numbers 208869-92-5 and 208869-94-7.	1-94
X	STN FILE CA ABSTRACT 123:331621 & F. BETSOU et al., Gene, 1995, 162(1), pp. 165-166. See abstract and CAS Registry number 170560-61-9.	1-94
X	STN FILE CA ABSTRACT 112:173135 & P. GLASER et al., Mol. Microbiol., 1988, 2(1), pp. 19-30. See abstract and CAS Registry number 126469-81-6.	1-94
X	STN 111:72007 & P. GLASER et al., EMBO J., 1988, 7(12), pp. 3997-4004. See abstract and CAS Registry 121889-91-6.	1-94

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/01190

Box I **Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos :

because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos : **1-94 (all in part)**

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

See attached sheet.

3. ☐ Claims Nos :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II **Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/01190

Supplemental Box
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No: I(2)

A subsequence search in the Registry file of the peptides defined by Claim 1 resulted in 2608 sequences that contained the defined sequences. This corresponded to 1381 Chemical Abstracts. Furthermore, this result does not take into account that the claims include variants and biologically active fragments of each of the individual peptides. Accordingly it is not economical to search this result.

Moreover, the invention appears to lie in the identification of new domains that are involved in Albicidin synthesis in X. albilineans. However, the domains defined in the claims constitute as few as four amino acids. Whilst this may be the active site of the domains in question, the function of a domain will be dependent on the constitution and topology of an entire region of the protein. Accordingly, the present search has been limited to the domains of the multifunctional polyketide-peptide synthase gene that appear to possess the functionalities defined in Claim 1, namely positions 1230-3116 (acyl-CoA ligase region); 3423-4724 and 9117-10367 (ketosynthase regions); 6660-7142 (ketoreductase region); 3117-3422, 8598-8795 and 8859-9068 (acyl carrier regions); 12447-14066 (adenylation region); 10890-11150 and 14067-14306 (peptidyl carrier regions); 11151-12446 and 14307-15632 (condensation regions). Similarly the search of the PPTase motifs have been limited to the specific peptides defines by SEQ ID NO 89, 93, 91, 87, 99, 101, 103, 105, 107 and 91. It is not possible and/or economically viable to search for variants and biologically acive fragments of these sequences.